Protective Effect of Spirulina Against Mitomycin C-Induced Genotoxic Damage in male Rats

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Abstract: *Spirulina platensis* (SP) is a filamentous cyanobacterium microalgae with potent dietary phyto-antioxidant, anti-inflammatory and anti-cancerous properties. The present study aimed to investigate the protective effect of Spirulina against Mitomycin C (MMC)-Induced genotoxic damage in male rats. To evaluate the protective role of *Spirulina platensis* expression alterations of the Bcl-2, CK8, CK19, p53, p21, and p27 genes and formation of micronucleus in male rats were investigated. Sixty Swiss albino male rats were divided into six groups. Group 1, animals were fed on a standard diet as untreated control group. Group 2 animals were fed on a standard diet mixed with 1% SP. Groups 3, animals were fed on a standard diet mixed with 1% SP powder followed by MMC (0.5 mg/kg). Group 4 animals were fed on a standard diet mixed with 1% SP powder followed by MMC (2 mg/kg). Groups 5 and 6 animals were fed on a standard diet followed by MMC (0.5 and 2 mg/kg, respectively. All the animals were sacrificed after an experimental period of 12 weeks. The expression of Bcl-2, CK8, CK19, p53, p21 and p27 genes was investigated using reverse transcription polymerase chain reaction (RT-PCR). The results revealed that MMC treatment induced expression alterations of genes related to apoptosis. Also MnPCEs formation was increased in bone marrow of male rats treated with MMC. These alterations of the gene expression as well as the MnPCEs formation were markedly suppressed when male rats were supplemented with SP for 12 weeks. Conclusion: These findings suggest that SP exerts its anti-mutagenic properties by inhibiting alterations in the gene expression and the MnPCEs formation in the hepatic tissues and bone marrow cells of male rats exposed to MMC.
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Keywords: *Spirulina platensis*, Mitomycin C, Gene expression; RT-PCR; Rats; MnPCEs formation.

Introduction

Mitomycin C (MMC) is a quinone-containing antibiotic originally isolated from Streptomyces caespitosus in 1958 (Wakaki et al.,). MMC has been used to treat a wide variety of solid tumors. Although current use of MMC is limited, this agent continues to be a key element in several clinical trials due to its intrinsic efficacy against many solid tumors and preferential activity in hypoxic tumoral cells Workman and Stratford (1993), MMC has a synergistic effect with radiotherapy via its radiosensitizing effects, targeting hypoxic cells in radiation resistant tumors, Sartorelli et al., (1994); Pors and Patterson(2005). To achieve its alkylating activity, MMC requires a bioreductive transformation to form active species that crosslink DNA, Dorr (1988); Na et al., (2001); Wang et al., (2007). Depending on the biotransformation pathway, metabolism of MMC may generate ROS Gustafson and Pritsos (1992). When ROS interact with cells and exceed endogenous antioxidant systems, there is indiscriminate damage to biological macromolecules such as nucleic acids, proteins, and lipids Offord et al., (2000).

Many plant-based chemopreventive agents are recognized to exert their anticarcinogenic effects by inhibiting cell proliferation and inducing cell differentiation and apoptosis. However, the chemo-preventive efficacies of these plants need to be tested in well established experimental animal tumour and genotoxic models, Subapriya et al., (2006) *Spirulina platensis* (SP) is a cyanobacterium being used in many countries as nutritional supplement for human and animal consumption. SP has been labelled as a powerful food, rich in proteins, carbohydrates, polyunsaturated fatty acids, sterols and some more vital elements like calcium, iron, zinc, magnesium, manganese and selenium. It is a natural source of vitamin B12, vitamin E, ascorbic acid, tocopherols and whole spectrum of natural mixed carotene and xanthophyll phytopigments Chamorro et al.,(1996); Piñero Estrada et al.,(2001); Chamorro et al.,(2002).

SP is well known for its anti-inflammatory and anti-cancerous properties. A hot water extract of SP has been orally administered to patients as an anti-

cancer and anti-viral agent. SP is best known as an immune booster by stimulating natural killer (NK) cells and co-operative action of IL-12 and IL-18 for NK-mediated IFN gamma production Hirahashi et al.,(2002).These SP-stimulated NK cells can fight illnesses other than cancer. SP hinders the growth of oral cancer. SP extract has been shown to inhibit tumor initiation in Syrian hamster cheek pouch mucosa painted with 7,12-dimethylbenz[a]anthracene Grawish (2008).Such an inhibitory effect may be attributed to the repair of carcinogen- damaged DNA, and SP has been suggested as an efficient radical scavenger, Romay et al.,(1998); Vadiraja et al.,(1998); Upasani et al.,(2001); Premkumar et al.,(2004).Other studies have reported that the unique polysaccharides of SP enhance cell nucleus enzyme activity and potentiate the process of DNA repair, Pang et al.,(1988); Kaji et al.,(2002).

Since cellular harm produced by MMC is thought to be at least partially due to a free radical mechanism, and MMC generates micronuclei-induced genotoxic damage in animal models, Hayashi et al.,(1992); Grisolia et al.,(2002) ,the aim of this work was to assess the genotoxic effect of MMC. These effects were measured as the number of micronucleated polychromatic erythrocytes (MN-PCE) from the bone marrow cells and the ability of MMC to induce alterations in gene expression of several genes related to cell apoptosis (Bcl-2, CK8, CK19, p53, p21 and p27) in hepatic tissues. We also assessed the potential protective action of SP against both micronuclei formation and changes in the gene expression due to MMC.

2. Materials and Methods

2.1. Materials:

2.1.1. Chemicals

Reagents and solvents used in the current study were of the highest possible grade available. The Mitomycin C was purchased from Sigma-Aldrich (USA). Reagents for RT-PCR method were purchased from Invitrogen (UK) and Fermentas (Germany).

2.1.2. Experimental Animals

Sixty Swiss albino male rats weighing 80-100 g were obtained from the Animal House at King Fahad Medical Research Centre, King Abdul Aziz University, Saudi Arabia. The animals were kept individually in wire bottomed cages at room temperature (25 ± 2 ºC) under 12 h dark-light cycle. They were maintained on standard laboratory diet and water *ad libitum*. The animals were allowed to acclimatize their new conditions for one week before commencing experiment, then they were distributed into eight groups (10 rats/ group). All animals received human care in compliance with the guidelines of the Ethical Committee of Medical Research, King Abdul Aziz University, Saudi Arabia.

2.1.3. Preparation of SP extract

SP algae used in this work was purchased from local market in Saudi Arabia. Then SP was cultured in our laboratory at King Abdul Aziz University, under optimal conditions on Zarrouk medium Andrade and Costa (2008). Algal mass was harvested every 3 weeks by continuous centrifuge, air dried and ground to powder form.

2.2. Methods:

2.2.1. Experimental design

After an acclimation period of one week, male albino rats 60-day-old (n=10 per group) were treated for 12 weeks and divided into the following groups: Group 1 (Untreated control group): animals were fed on a standard diet and given water *ad. libitum* for 12 weeks; Group 2 (SP-treated group): animals were fed on a standard diet mixed with 1% SP powder, and given water *ad. libtium* for 12 weeks; Group 3 (SP-MMC_1-treated group): animals were fed on a standard diet mixed with 1% SP powder, and given water *ad. libtium* for 12 weeks followed by MMC (0.5 mg/kg) dissolved in saline and injected intraperitoneally in a single dose 24h prior to sacrifice; Group 4 (SP-MMC_2-treated group): animals were fed on a standard diet mixed with 1% SP powder, and given water *ad. libtium* for 12 weeks followed by MMC (2 mg/kg) dissolved in saline and injected intraperitoneally in a single dose 24h prior to sacrifice; Group 5 (MMC_1-treated group): animals were fed on a standard diet and given water *ad. libtium* for 12 weeks followed by MMC (0.5 mg/kg) dissolved in saline and injected intraperitoneally in a single dose 24h prior to sacrifice; Group 6 (MMC_2-treated group): animals were fed on a standard diet and given water *ad. libtium* for 12 weeks followed by MMC (2 mg/kg) dissolved in saline and injected intraperitoneally in a single dose 24h prior to sacrifice.

During treatment, animals were observed twice daily for signs of moribundity and mortality. Body weights were recorded initially, once weekly, and at termination. At the end of the experimental period, the animals were rapidly sacrificed and the samples of the liver tissues and bone marrow cells of each animal were taken for gene expression and micronucleus analyses, respectively. Liver tissues were snap-frozen in liquid nitrogen and were kept at -80°C until analysis

2.2.2. Micronucleus test

The bone marrow cells resuspended in a small volume of fetal calf serum on a glass slide were used for smear preparation. The smear of bone marrow cells was prepared from each rat. After air-drying, the slide was fixed in methyl alcohol for 10 min and stained with 5% Giemsa stain for 10 min. Three slides were prepared for each animal and were coded before observation and one was selected for scoring. From each coded slide, 3,000 polychromatic erythrocytes (PCEs) were scored for the presence or micronuclei under oil immersion at high power magnification. In addition, the percentage of micronucleated polychromatic erythrocytes (%MnPCEs) was calculated on the basis of the ratio of MnPCEs to PCEs, Adler (1984)

2.2.3. Semi-quantitative Reverse Transcription-PCR

2.2.3.1. RNA extraction

Stored liver tissue samples (at −80°C prior to extraction), were used to extract the total RNA. Total RNA was isolated from 100 mg of tissues by the standard TRIzol extraction method (Invitrogen, UK) and recovered in 100 µl molecular biology grade water. In order to remove any possible genomic DNA contamination, the total RNA samples were pre-treated using DNA-free™ DNase treatment and removal reagents kit (Ambion, Austin, TX, USA) following the manufacturer's protocol. The RNA concentration was determined by spectrophotometric absorption at 260 nm.

2.2.3.2. Synthesize of First-strand cDNA

To synthesize the first-strand cDNA, 5 µg of the complete Poly(A)$^+$ RNA isolated from rat samples was reverse transcribed into cDNA in a total volume of 20 µl using 1 µl oligo (poly(deoxythymidine)$_{18}$) primer, El-Makawy, et al;,(2008) . The composition of the reaction mixture consisted of 50 mM MgCl2, 10x reverse transcription (RT) buffer (50 mM KCl; 10 mM Tris-HCl; pH 8.3), 200 U/ µl reverse transcriptase (RNase H free), 10 mM of each dNTP, and 50 µM of oligo(dT) primer. The RT reaction was carried out at 25°C for 10 min, followed by 1 h at 42°C, and finished with denaturation step at 99°C for 5 min. Afterwards the reaction tubes containing RT preparations were flash-cooled in an ice chamber until being used for DNA amplification through polymerase chain reaction (PCR) Ali et al;,(2008).

2.2.3.3. RT-PCR assay

The first strand cDNA from different mammary tissue samples was used as templates for the semi-quantitative RT-PCR with a pair of specific primers in a 25-µl reaction volume. The sequences of specific primer and product sizes are listed in Table 1. β-Actin was used as a housekeeping gene for normalizing mRNA levels of the target genes. The reaction mixture for RT-PCR was consisted of 10 mM dNTP's, 50 mM MgCl2, 10x PCR buffer (50 mM KCl; 20 mM Tris-HCl; pH 8.3), 1U/ µl taq polymerase, and autoclaved water. The PCR cycling parameters of the studied genes (Bcl-2, CK8, CK19, p53, p21 and p27) were performed as the PCR condition summarized in Table 1. The PCR products were then loaded onto 2.0% agarose gel, with PCR products derived from β-actin of the different rat samples**.** Each reaction of the RT-PCR was repeated with ten rats, generating new cDNA products at least ten times per each group.

2.2.4. Statistical Analysis:

All data were analyzed using the General Liner Models (GLM) procedure of Statistical Analysis System, SAS, 1982. followed by Scheffé-test to assess significant differences between groups. The values are expressed as mean ± SEM. All statements of significant were based on probability of $P < 0.05$.

Table I. Primers and PCR thermocycling parameters

Primer	Sequence (5′-3′)	PCR conditions	RT-PCR (bp)
Bcl-2	GGT GCC ACC TGT GGT CCA CCT G CTT CAC TTG TGG CCC AGA TAG G	42°C for 1 h, 95°C for 15 min, 32 cycles of (i) 94°C for 30 s, (ii) 62°C for 30 min, (iii) 72°C for 1 min and 72°C for 10 min	376
Cytokeratin 8	TTCCTGGAGCAGCAGAACAA	25 cycles: 94°C, 30 s; 65°C, 30 s;	255

CK8	GAGG ACAAATTCGTTCTCCAT	68°C, 1 min Final extension: 68°C, 2 min	
Cytokeratin 19 CK19	A TTCTTGG TGCCACCATTGA TCCTCATGGTTCTTC TTCAGG	30 cycles: 94°C, 30 s; 65°C, 30 s; 72°C, 1 min Final extension: 72°C, 2 min	238
p53	CGCAAAAGAAGAAGCCACTA TCCACTCTGGGCATCCTT	25 cycles: 94°C, 30 s; 65°C, 30 s; 68°C, 1 min Final extension: 68°C, 2 min	118
p21	ACCTCTCAGGGCCGAAAAC TAGGGCTTCCTCTTGGAGAA	25 cycles: 94°C, 30 s; 65°C, 30 s; 68°C, 1 min Final extension: 68°C, 2 min	88
p27	CAGAGGACACACACTTGGTAGA TCTTTTGTTTTGAGGAGAGGAA	35 cycles: 93°C, 30 s; 56°C, 45 s; 74°C, 45 s Final extension: 74°C, 10 min	124
β-Actin	GTGGGCCGCTCTAGGCACCAA CTCTTTGATGTCACGCACGATTTC	25 cycles: 94°C, 30 s; 65°C, 30 s; 68°C, 1 min Final extension: 68°C, 2 min	540

3. Results

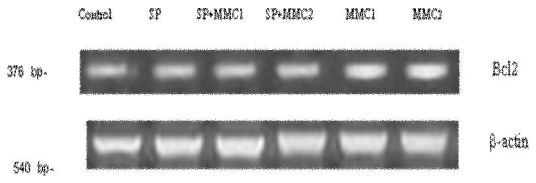
3.1. Rat survival and body weight

The results revealed that no significant differences in survival were observed between the untreated control, SP, and SP+MMC groups, with approximately 98% of the animals surviving to study termination (range = 92–99%). However, the survival rate between MMC animals was relatively decreased compared with control which reached 86%. The mean body weights of rats receiving SP or SP+MMC did not significantly differ from controls over time. However, the mean body weight of rats exposed to MMC at low (0.5 mg/kg) or high dose (2 mg/kg) was only 85% and 81% that of controls by the end of the study, respectively.

3.2. Semi-quantitative RT-PCR

Reverse transcription polymerase chain reaction was conducted to verify the expression levels of the Bcl-2, CK8, CK19, P53, P21, and P27 genes related to cell apoptosis in liver tissues of male rats (Table 1). Supplemented with SP for 12 weeks and exposed to several doses of MC 24h prior to sacrifice.

The results of the present study revealed that expression level of Bcl2 gene was significantly higher in hepatic tissues of MMC groups than control and other SP groups (Fig. 1). However, the expression level of Bcl2 gene in SP treatment was significantly lower compared with MMC groups and was similar to control group. Moreover, this expression level was significantly also lower in SP plus the low and high does of MMC groups than MMC groups. On the other hand, the Bcl2 expression in 0.5 mg/kg of MMC group was lower than the 2 mg/kg of MMC group (Fig. 1).



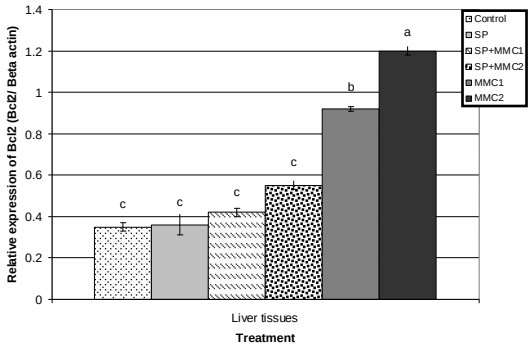


Figure 1: Semi-quantitative RT-PCR confirmation of Bcl2 gene in liver tissues of male rats treated with Spirulina (SP, for 12 weeks) with or without mitomycin C (MMC, at 24h prior to sacrifice) (a&b). Within each column means superscripts with different letters are significantly different (P ≤ 0.05).

The expression level of CK8 and CK19 genes in the hepatic tissues of male rats exposed to MMC at the low and high doses was significantly higher than control and other SP groups (Fig. 2 and 3). Moreover, the expression level of this gene in both MMC groups was relatively similar. However, these genes showed expression level significantly lower in SP plus MMC groups than MMC group alone (Fig. 2 and 3).

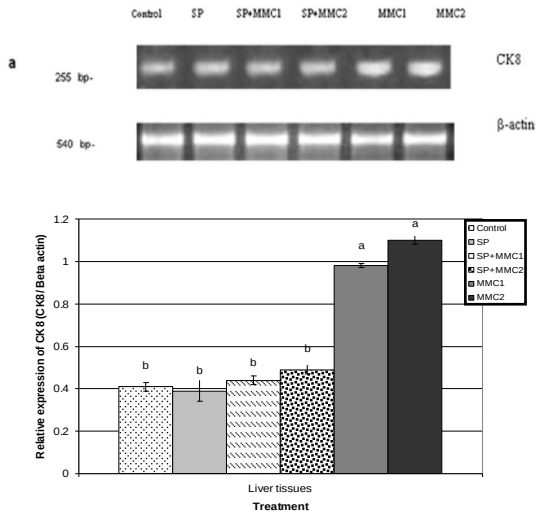


Figure 2: Semi-quantitative RT-PCR confirmation of CK8 gene in liver tissues of male rats treated with Spirulina (SP, for 12 weeks) and mitomycin C (MMC, at 24h prior to sacrifice) (a&b). Within each column means superscripts with different letters are significantly different (P ≤ 0.05).

From the determination of the expression level of gene p53 the results revealed that the expression level was significantly higher in hepatic tissue of MMC group than control and other SP groups (Fig. 4). However, the expression level of p53 gene in 2 mg/kg of MMC group was significantly higher than 0.5 mg/kg of MMC group. In contrast, this expression was significantly lower in SP plus 0.5 or 2 mg/kg of MMC groups than MMC groups (Fig. 4).

The expression level of p21 gene in the hepatic tissues of male rats exposed to MMC at the low and high doses was significantly higher than control and other groups (Fig. 5). However, the expression of p21 gene showed level of expression did not significantly change in SP plus 0.5 or SP plus 2 mg/kg of MMC compared with MMC groups. In the same trend, the p21 expression in SP alone was similar to control group (Fig.5).

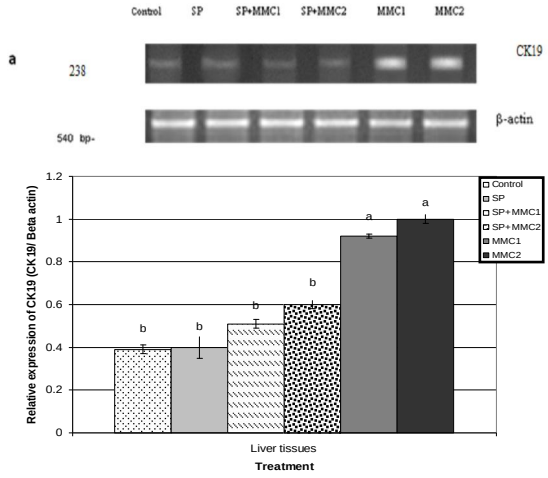


Figure 3: Semi-quantitative RT-PCR confirmation of CK19 gene in liver tissues of male rats treated with Spirulina (SP, for 12 weeks) and mitomycin C (MMC, at 24h prior to sacrifice) (a&b). Within each column means superscripts with different letters are significantly different (P ≤ 0.05)

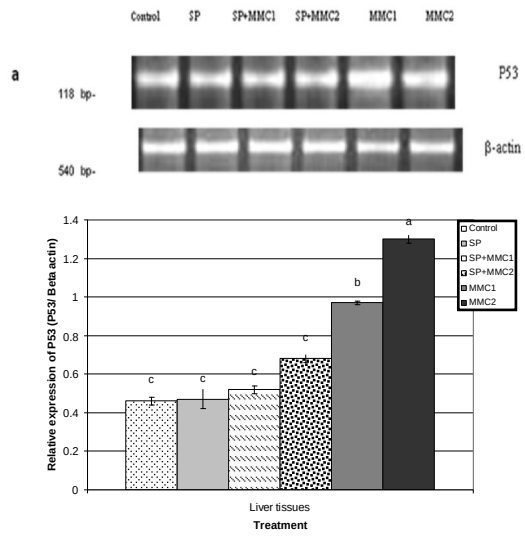


Figure 4: Semi-quantitative RT-PCR confirmation of P53 gene in liver tissues of male rats treated with Spirulina (SP, for 12 weeks) and mitomycin C (MMC, at 24h prior to sacrifice) (a&b). Within each column means superscripts with different letters are significantly different (P ≤ 0.05).

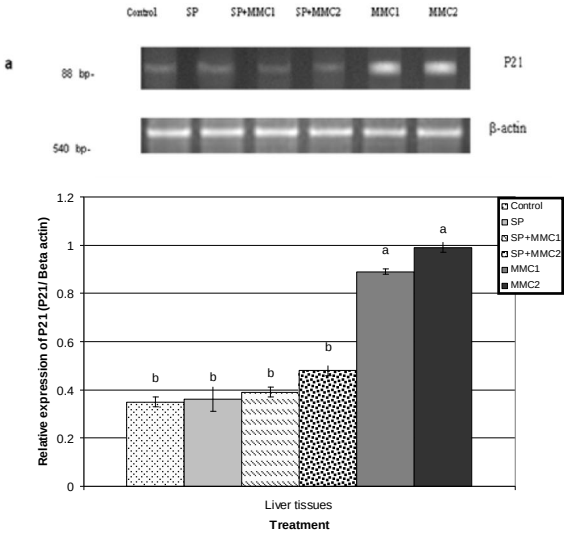


Figure 5: Semi-quantitative RT-PCR confirmation of P21 gene in liver tissues of male rats treated with Spirulina (SP, for 12 weeks) and mitomycin C (MMC, at 24h prior to sacrifice) (a&b). Within each column means superscripts with different letters are significantly different (P ≤ 0.05).

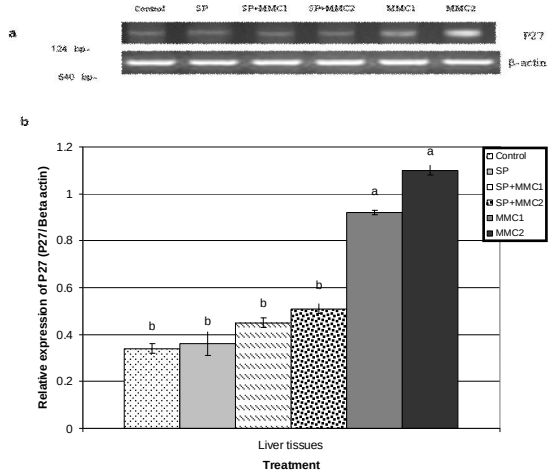


Figure 6: Semi-quantitative RT-PCR confirmation of P27 gene in liver tissues of male rats treated with Spirulina (SP, for 12 weeks) and mitomycin C (MMC, at 24h prior to sacrifice) (a&b). Within each column means superscripts with different letters are significantly different (P ≤ 0.05).

The expression profile of p27 gene was significantly higher in the hepatic tissues of male rats exposed to MMC at the low and high doses than control and other groups (Fig. 6). However, the expression level of p27 gene in SP plus all doses of MMC groups was not significantly different compared with control group. Also, this expression in SP alone was similar to control group (Fig. 6).

Table 2. Micronucleated polychromatic erythrocytes (MnPCEs) of male rats treated with Spirulina (SP, for 12 weeks) and mitomycin C (MMC, at 24h prior to sacrifice) (mean ± SEM).

Treatment	Conc. (mg/kg)	MnPCEs / 3000 PCEs
Control	---	8.1 ± 0.2^{c}
SP		8.9 ± 0.9^{c}
SP+MMC1*	1%+0.5	9.4 ± 0.8^{c}
SP+MMC2*	1%+2.0	10.2 ± 0.7^{c}
MMC1*	0.5	18.7 ± 1.1^{b}
MMC2*	2.0	24.3 ± 1.0^{a}

*: **Mitomycin C and MAA-QD were injected as a single dose and then the bone marrow cells were collected 24h after injection;** ^{a,b,c} **values with different superscripts within columns represent significant statistical differences (P< 0.05, Scheffé-Test).** ^{a,b,c} **values with similar superscripts within columns represent no significant statistical differences (P < 0.05, Scheffé-Test).**

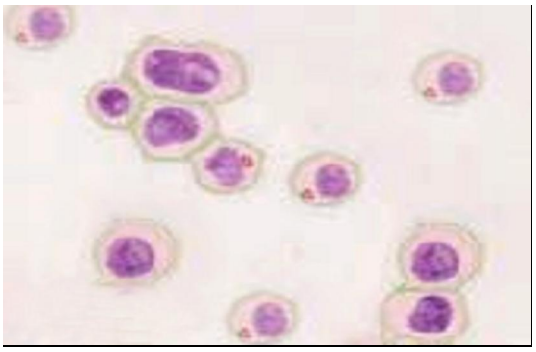


Fig. 7. Bone marrow erythrocytes of male rats treated with mitomycin C (MMC, at 24h prior to sacrifice). Arrow: micronucleated polychromatic erythrocyte (MnPCEs).

3.3. Micronucleus assay

Effect of SP and MMC on MnPCEs formation in the bone marrow cells of male rats is summarized in Table (2) and Figure (7). The results revealed that after SP supplementation for 12 weeks MnPCEs formation in the bone marrow cells was relatively similar to those in control group.

Treatment of male rats with 0.5 mg/kg of MMC at 24h before sacrifice increased significantly the formation of MnPCEs. Whereas, the formation of MnPCEs increased in the 0.5 mg/kg of MMC group to 230.7% compared to control group. In addition, MnPCEs formation in the 2 mg/kg of MMC group was significantly higher than all other group. Whereas, the formation of MnPCEs increased in the 2 mg/kg of MMC group to 300% compared to control group (Table 2).

On the other hand, exposure of male rats with SP plus low and high dose of MMC did not significantly increase the incidence of MnPCEs compared with control group. Whereas, the formation of MnPCEs in the bone marrow cells of these groups was relatively similar to control group (Table 2).

4. Discussion

The current work was carried out to investigate the effect of MMC on the expression alterations of the genes (Bcl-2, CK8, CK19, P53, P21 and P27) related to apoptosis and formation of Mn PCEs in male rats. Also the protective role of SP was determined to inhibit the alterations in the gene expression and suppress the Mn PCEs formation in male rats.

This study was associated with over-expression of the all tested genes in the MMC treated rats. These results were in great agreement of the results reported by Emi et al. (2005), who found that therapy treatment with doxorubicin (DOX), mitomycin C (MMC) on the human breast cancer cell line altered the expression level of several apoptosis genes. This was associated with over-expression of Bcl-2 and Bcl-xL genes in breast cancer cell line. Our results showed also over-expression of Bcl-2, p53, p21, p27, CK8 and CK19 genes in the liver tissues of male rats treated with MMC.

Bcl-2, a prominent anti-apoptotic member of the Bcl-2 family inhibits the release of pro-apoptotic molecules and cytochrome C from the mitochondria thereby inhibiting apoptosis and permitting persistence of tumour cells. In addition, over-expression of both p53 and Bcl-2 has been reported to inhibit transcriptional activation of Bax gene, Lucken-Ardjomande andMartinou(2005); Cavalier et al. (2005). Thus, over-expression of Bcl-2, p53, p21, p27, CK8, and CK19 genes in the liver tissues may confer a selective growth advantage on hepatic cells.

Administration of SP reduced the incidence of MMC induced rat hepatic tissues genetic alteration. Whereas, the SP was able significantly to down-regulate Bcl-2, p53, p21, p27, CK8 and CK19 expression in the hepatic cells at the dose of 1% of the rat diet. These results agreed the results of Ismail et al. (2009), who found that SP significantly down-regulated Bcl-2, PCNA and p53 expression in the liver of dibutyl nitrosamine (DBN)-treated rats.

The results of the present study substantiate the anti-genetic alteration properties of SP reported in literature. Polysaccharide extract from *Spirulina platensis* is a potent inhibitor of corneal neovascularization (CNV), decreased the expression of serine threonine kinase (AKT) and extracellular regulated kinase1/2 (ERK1/2) genes and that it may be of benefit in the therapy of corneal diseases involving neovascularization and inflammation Yang et al. (2009).

Roy et al. (2007) reported that reactive oxygen species (ROS)- levels determined in mouse macrophage cell line showed that C-Phycocyanin (C-PC), a biliprotein from Spirulina platensis was more effective in reduction of multidrug resistance (MDR1) gene expression. These results suggest that 2-acetylaminofluorene (2-AAF) induces MDR1 by ROS dependent pathway and C-PC is a potential a regulator of MDR1 expression.

To understand the regulation of the p53, p21 and p27 in the present study that it is known to induce apoptosis through inhibition of Bcl-2, amplification of death signals and activation of caspases, Haupt et al. (2003) . Mutations in p53 have been reported to occur in 40% of all human tumors. While wild type p53 functions as a TSG, mutant p53 functions as an oncogene. Mutant p53 protein is reported to have lost the ability to act as a growth suppressor and gained the ability to promote cell

proliferation. Over-expression of p53, p21 and p27 may enhance genetic instability by facilitating cell proliferation and inhibiting DNA repair and apoptosis. Furthermore, p53 activates telomerase and factors involved in angiogenesis as well as metastasis. In particular, p53 mutations have been reported to be associated with over-expression of Bcl-2, Haupt et al. (2003); Cavalier et al. (2005).

The present study clearly demonstrates that inhibition of cell proliferation and induction of differentiation and apoptosis may be major mechanisms through which SP exerts its anti-genotoxicity and anti-carcinogenic properties. This is the first report of the *in vivo* chemo-preventive effect of SP against MMC-induced rat liver genotoxicity and suggesting its potential use in chemoprevention of genetic alteration.

The investigation performed by Ismail et al. (2009), found that SP inhibited the incidence of liver carcinogenesis and prevented the expression levels of proliferating cell nuclear antigen (PCNA) and p53 were highly elevated in the liver of DBN-treated rats, but were significantly reduced by SP supplementation.

SP has been traditionally used for nutrition worldwide by people from Mexico, Africa and Asia. It is being widely studied for its possible antioxidant, antibacterial, and antiparasitic properties, and for several medical conditions such as allergies, ulcers, anaemia, heavy-metal poisoning, and radiation poisoning, Vadiraja et al.,(1998); Pors and Patterson(2005). SP or its extracts can prevent or inhibit cancer in humans and animals, Pang et al.,(1988); Schwartz and Shklar (1987); Mathew et al.,(1995); Qureshi et al.,(1996); Qureshi et al.,(1996);Chamorro et al.,(1996); Piñero Estrada et al.,(2001); Chamorro et al.,(2002); Kaji et al.,(2002).

In the present study, SP alone did not cause any side effects or organ toxicity, but it was remarkably effective in reducing the incidence of gene expression changes in the liver and MnPCEs formation in the bone marrow cells caused by MMC, suggesting its potential therapeutic effect in our model.

Mathew et al.,(1995) reported a chemopreventive role of SP against oral cancer. It has been suggested that the ability of SP to inhibit carcinogenesis is due to its anti- oxidant properties that protect tissues from cell damage, Khan et al. (2005). The potential hepatoprotective role of SP may be associated with its antioxidant constituents such as selenium, chlorophyll, carotene, gamma-linolenic acids, tocopherol, phenolic compounds content and vitamin E and C working individually or in synergy Kay (1991); Torres-Duran et al., (1999); Kaushik et al.,(2001); García-Martínez et al., (2007).SP has been shown to be effective against free radical induced cellular transformation, Romay et al.,(1998); Upasani et al.,(2001). In addition, phycocyanin, the main pigment present in SP, can inhibit cytochrome P450 mediated reactions involved in the formation of reactive metabolites of the hepatotoxins, Vadiraja et al.,(1998). Mittal et al. showed that SP significantly reduced the hepatic cytochrome P-450 content and significantly induced the hepatic glutathione S-transferase activity, Mittal et al.,(1999).

Moreover, Ismail et al. (2009), reported that *in vitro* studies revealed that polysaccharides of SP enhanced cell nucleus enzyme activity and DNA repair mechanisms, which are known to be closely associated with chemoprevention properties of natural products.

In the present study, liver and bone marrow cells of both control and SP did not express Bcl-2, p53, p21, p27, CK8, and CK19 genes and did not form the MN, respectively. However, liver sections of rats treated with MMC showed significant increase in the gene expression and MnPCEs formation, which was reduced by SP supplementation. This might be attributed to the anti-mutagenic effect of SP which minimized DNA damage caused by MMC.

Oxidative stress and chronic inflammation are closely associated with increased risk of cancer Wang et al.,(2002).. High concentrations of nitric oxide (NO) products generated by MMC can cause DNA damage, either directly or through secondary molecules, by nitroso- active deamination, DNA strand breakage, and DNA modifications Ambs et al.,(1997). NO-induced DNA damage can lead to p53 accumulation and p53-mediated apoptosis, Forrester et al.,(1996); Messmer and Brune (1996).

In summary, our study is the first to show that MMC-induced changes in the gene expression and MnPCEs formation in rat liver and bone marrow cells which were prevented by SP supplementation, suggesting that SP is a protective phyto-antioxidant against liver and bone marrow toxicity and an anti-genotoxic agent.

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