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Potential Modulator Role of *Chlorella vulgaris* and *Spirulina platensis* On Monosodium Glutamate Oxidative Stress, Genotoxicity, Apoptotic Gene Expression and Histopathological Alterations

[1]Aida I El makawy*, [1]Sekena H Abdel-Aziem, [2]Faten M. Ibrahim, [3]Hafiza A Sharaf, [1]Omaima M Abd-Elmoneim, [1]Ahmed M Darwish

[1]Cell Biology Dept., [2]Medicinal and Aromatic Plants Research Dept., [3]Pathology Dept., National Research Centre, 33 El Bohouth St., Dokki, Giza, Egypt.
*Corresponding author: aelmakawy@yahoo.com

Abstract: *Chlorella vulgaris* (*CH*) and *Spirulina platensis* (*SP*) are microalgae being touted as functional foods with a number of health benefits. *CH* and *SP* have potent antioxidant activity and provoke a free radical scavenging enzyme system. The present study was sought to assess the potential protective activities of *CH* and *SP* aqueous extracts against MSG toxicity in male mice. Mice were orally gavages with MSG at dose of (1200 mg/kg b.w) alone or with either *CH* or *SP* (500mg/kg b.w) daily for 28 days. Algae total phenolic and flavonoids content were measured, it was noticed that Sp have the higher total phenolic content while *CH* have the high flavonoids content. Bone marrow and liver were collected for genetical, biochemical, histological and histochemical analysis. The results appeared that MSG increased lipid peroxidation, apoptosis, DNA fragmentation and decreased hepatic glutathione concentration and total antioxidant activities. In addition, it is up regulating mRNA Bax and caspase-3genes, down-regulating Bcl-2 genes expression. Moreover, MSG showed liver histopathological alterations and increased the liver DNA content. In contrast, co-treatment of MSG plus either *CH* or *SP* succeeded to normalize the biochemical parameters levels, reduced the cytotoxicity, genotoxicity and ameliorated the hepatic histopathological and histochemical alterations. Therefore, *CH* and *SP* aqueous extracts have protective efficiency due to their content of bioactive compounds and could be considered as available resource of natural antioxidants.
Keywords: *Spirulina platensis, Chlorella vulgaris,* MSG, biochemical parameters, genotoxicity, apoptosis, gene expressions, histopathological and histochemical.

Introduction

Various environmental chemicals, industrial pollutants and food additives have been concerned as causing harmful effects by reacting with DNA causing alteration through the production of ROS [1]. Excessive ROS production has been known to stimulate signaling cascades causing apoptosis [2]. Apoptosis acting a vital role in the development of tissue homeostasis and can get rid of damaged cells that are potentially hurtful to the body [3].

Monosodium glutamate (MSG) is the sodium salt of a naturally occurring glutamic acid [4]. Monosodium glutamate is a food additive used to increase palatability. This popular taste enhancer commonly used in flavored chips, snacks, soups or sauces, tuna, frozen foods and manufactured meats [5]. Several researches in animals have indicated that MSG is toxic to the various organs [6,7,8]. It caused alterations in the levels of lipids, proteins, cholesterol and thiobarbituric acid reactive substances (TBARS) [9,10]. Several studies

confirmed that MSG induced hyperlipidemia, hyperglycemia and oxidative stress [11] and it has widely incriminated by various researchers for its neurotoxic effect[12]. In addition, El Makawy and Abdou [13] reported that MSG induced genetic materials damage. The mechanisms by which glutamate-induced toxicity is not understood although much evidence reported that MSG toxicity involves oxidative stress. Generation of ROS in different body cells is known to induce damage to DNA and lipid peroxidation in cellular membrane due to damage of the polyunsaturated fatty acids lead to apoptotic cellular death [6].

Although the defense system of human body consisting of antioxidants to eliminate the negative effects of reactive agents, but the accumulated free radicals over the human life weaken the efficiency of their immune system. It has known that this can be defeated by supplementation of exogenous antioxidants through diet [14]. Marine microalgae are photosynthetic microorganisms and received special interest for food supplement use by pharmaceutical and food industries [15]. Chlorella *vulgaris (CH)*, unicellular green algae has been broadly used as food supplement and credited with high antioxidant and therapeutic abilities [16]. Chlorella contains high content of protein and fibers, beside, much kind of vitamins and minerals. It is also wealthy in chlorophylls, lutein, and β-carotene. Many health-promoting benefits as oxidative stress and lipid lowering has been report in animals and humans [17,18,19]. Moreover, as *CH* exhibits high antioxidant activity, its radical scavenging property has been manipulated to promote its anticancer and antiatherogenic properties[20,21]. In addition, *CH* has been shown to own many biological property such as promote the growth rate of animals [22], modulating immune function [23 ,24], preventing dyslipidemia, and ameliorating hyperglycemic status of diabetes induced by streptozocin [25, 26]. Furthermore, *CH* is commonly applied as a vital item in the food industry [27].

Spirulina platensis (SP) blue - green algae due to the occurrence of chlorophyll and phycocyanin pigments in its cellular structure. It is the mainly familiar and broadly used species and has been widely studied in medicine and food industry [28,29]. It contains very powerful naturally occurring antioxidant and used as nutritional supplement for human and animal consumption [30]. Besides, its active ingredient; Cphycocyanin reveal antiinflammatory, neuroprotective, hepatoprotective, immunomodulatory and anticancer properties[31]. In addition, *SP* has been provided organs protection against many drugs and chemicals induced toxic injuries [32,33,34.] Moreover, many studies are interested with the *Spirulina* prospective antioxidant activity. *Spirulina* strongly induces antioxidant enzyme activity that helps to thwart lipid peroxidation, DNA damage, and scavenges free radicals [35].

Therefore, the target of the current study was to estimate the potential protective role of *CH* and *SP* aqueous extracts against MSG toxicity in mice. The studies were conducted using (1): Biochemical analysis to determine the content of lipid peroxidation, glutathione reduced concentration (GSH) and total antioxidant activity in liver tissue of male mice. (2): Genetically evaluations to determine DNA damage and apoptosis by micronucleus test and AO/EB dual stain, DNA fragmentation, apoptosis and apoptotic gene expression in bone marrow and liver cells. (3): Histopathological and histochemical studies to examine the alteration in hepatocytes of male mice.

Materials and Methods

Chemicals

Monosodium glutamate(C5H9NO4·Na) with purity 99% NT Was sold in most open markets under the license of Ajinomoto Co. Inc., Tokyo, Japan. *Chlorella vulgaris* and *Spirulina platensis* powder were obtained from Algal Biotechnology Unit, National Research Centre (Dokki, Cairo, Egypt). Folin-ciocalteau reagent, gallic acid, querectin, $NaNO_2$, Na_2CO_3, $AlCl_3$ (Merck products), Glutathione reduced (GSH), malondialdehyde (MDA) and total antioxidant capacity were purchased from Biodiagnostic Company, Dokki, Giza, Egypt. All chemical reagents and solvents were of analytical grades and purchased from Sigma Chemical Co. (St. Louis, MO, USA).

Algae extract

Dried algae powder (500 g) was extracted with 2L of distilled water and left to stand for 48 hours at room temperature. The extract was filtered with Whatman No. 1 filter paper. The crude aqueous extract was concentrated using rotary evaporator under reduced pressure at 45^{o}C then the concentrated extracts were lyophilized and kept at -20°C.

Determination of total phenolic and flavonoids contents in the algae extracts

Total phenolic compounds and total flavonoids assay was conducted using the modified method of Marinova et al [36]. Powdered algae (0.5gm) were extracted using 50 ml of 80% aqueous methanol on an ultrasonic bath for 20min. Then the mixture was centrifuged for 5 min at 14,000 rpm. The supernatant was collected and used for quantification of total phenolic and flavonoids compounds.

Measurement of total phenolic content

Total phenolic (TP) compounds were assayed using the Folin-Ciocalteu assay 1mL extract or standard solution of gallic acid (20, 40, 60, 80,100mg/l). Then 1 ml of Folin-Ciocalteu's phenol reagent was added and the mixture was mixed and shaken. After 5 min 10 ml of 7% sodium carbonate was added, the mixture was then completed to 25 ml with distilled water mixed and allowed to stand at room temperature for 90 min. The absorbance against the prepared reagent blank was determined at 750 nm. using a spectrophotometer (Jasco V630 spectrophotometer). TP content was expressed as mg gallic acid equivalents (GAE/g extract).

Determination of total flavonoid content

Total flavonoid (TF) was determined using the aluminum chloride colorimetric method. 1ml of algae extract or standard solution of querectin (20, 40, 60, 80, 100mg/l) was added to 4 ml distilled water in a 10 ml flask. 0.3 ml of 5% $NaNO_2$ was added after 5 min 0.3 ml of 10% $AlCl_3$ was added and left for 6 min then 2 ml of 1 M NaOH was added. The mixture was diluted to 10 ml with distilled water. The absorbance of the solution was measured at 510 nm using a spectrophotometer (Jasco V630 spectrophotometer). The results were expressed as mg querecetin equivalents (Qu)/g extract all samples were analyzed in triplicate.

The acute oral toxicity test for algae extracts

The acute toxicity was estimated to evaluate any possible toxicity. Female albino mice (n=8) were tested by administering different extracts doses by increasing or decreasing the dose, according to the response of animal [37]. The dosing patron was 500, 1000, 1500, 2000, 2500, 3000, 3500, 4000, 4500 and 5000 mg/kg body weight for *Chlorella vulgaris (CH*) and *Spirulina platensis* (*SP*) aqueous extracts, while control group received only the normal saline. All groups were observed for any gross effect or mortality during 48hr. Death of half of examined animals was observed at 5000 mg/kg b.wt for aqueous extracts of both algae extracts. So, 500mg/kg (1/10th of 5000mg/kg) was selected as the maximum safety dose.

Animals

Adult Swiss albino male mice with average weight of 25± 5g were obtained from National Research Centre animal care center. Animals were housed in polypropylene cages under standard hygienic conditions of humidity (60 - 70%), temperature (25 ± 2°C) and a controlled 12 h light/dark cycle and were fed with rodent chow and water *ad labium*. Animals were acclimatized for 7 days to the experimental animal room conditions and in order to optimize treatment doses, all animals were fasted for 1 h prior to treatment administration. The research was approved by the Institutional Animal Ethics Committee for Care and Use of Laboratory Animals for Medical Research, National Research Center.

Experimental Design

Animals were randomly assigned into sex groups, each consist of eight mice and were gavages with 0.5ml of different solution used in the experiment. The first group used as control and was administered saline solution (0.5ml/ day/ 28 days). The second and third groups were the healthy normal groups that were administered aqueous extracts of medicinal algae extracts (1/10 LD50 =500 mg / kg /day/ 28 days), the fourth group was gavages with MSG dissolved in distilled water at recommended dose of 1200 mg/kg b.wt / day / 28

days [13]. The fifth and sixth groups were orally administered with MSG concurrently with *CH* and *SP* extracts in the same manner for 28days.

Biochemical Parameters

Liver homogenate

At the end of the experiment, Portions of liver from all animals in each group were homogenized in 50 mM Tris–HCl buffer (pH 7.4) containing 1.15 % potassium chloride by using potter-Elvehjem homogenizer with Teflon pestle. The homogenates were centrifuged at 10,000 g for 15 min at 4 °C. The collected supernatants were used for the estimation of the activities of enzymes.

Measurement of lipid peroxidation

Malondyaldehyde (MDA) determination in liver was assayed by Spectrophotometric method at 534nm Ohkawa [38] using thiobarbituric acid reaction.

Determination of glutathione reduced (GSH)

Reduced glutathione (GSH) was estimated by its reaction with 5, 5'-dithiobis (2-nitrobenzoic acid) (DTNB) to produce a yellow colored complex with absorption at 405 nm according to Beutler *et al* [39].

Determination of total antioxidant activity

The determination of the total antioxidant activity in liver was determined according to Koracevic *et al*[40].

Determine of DNA damage by micronucleus (MN) assay

Bone marrow slides were prepared according to the method of Krishna and Hayashi [41]. Bone marrow was flushed from both femurs with fetal calf serum into a Microfuge tube using a 1 ml syringe. Marrow was gently pulled up and down the syringe to prepare a uniform cell suspension. The smears were prepared and allowed to air dry, prior to fixation with methanol, and staining with MayGrunwald / Giemsa protocol. To evaluate the frequency of micronucleus, 2000 PCE per each mouse were analyzed for the presence of micronuclei by light microscopy. To evaluate the toxicity of bone marrow, the ratio of polychromatic erythrocytes to normochromatic erythrocytes (PCE/NCE) was calculated by counting 500 erythrocytes.

Determination of apoptosis by dual AO/EB fluorescent staining

Acridine orange/ethidium bromide (AO/EB) double fluorescent stain used to observe morphological changes in bone marrow cells as described by Liu *et al* [42]. Briefly, bone marrow was suspended in fetal calf serum and smeared on clean glass slides. Dual fluorescent staining solution (1µl) containing 100µg/ml AO and 100µg/ml EB (AO/EB, Sigma, St. Louis, MO) was added to suspension and then covered with a cover slip. The morphology of apoptotic cells was examined and 500 cells were counted within 20 min using a fluorescent microscope (Olympus, Japan). Dual AO/EB staining method was repeated 3 times at least. The living cells appear with normal green nucleus and apoptotic cells with orange- red stained nuclei. In each group, 500 cells were examined under fluorescent microscope using B2A filter and the apoptotic ratio was calculated as number of apoptotic cells /total cell count (×100).

DNA Fragmentation assays for apoptosis protocol

DNA fragmentation in liver tissues carried out according to Perandones *et al* [43]. In brief, about 10-20 mg of liver tissues were grinded in 400 µl hypotonic lysis buffer (10 mM Tris base, 1 mM EDTA and 0.2 % Triton X-10) and the cell was centrifuged at 11,000 rpm for 15 min at 4 ºC. The supernatant containing small DNA fragments were treated with equal volumes of absolute isopropyl alcohol and 0.5M NaCl, to precipitate the DNA, stored at -20ºC overnight and centrifuged at 11,000 rpm for 15 min at 4ºC. The pellet was washed with 200 µl of 70 % ethanol and allowed to dry at room temperature. Extracted DNA was reconstituted in 12 µl of Tris-EDTA buffer and 3µl loading buffer. The samples were incubated at 37°C for 20 min then electrophoresed on 1% agarose gels containing 0.71 µg/ml ethidium bromides. At the end of the runs, gels were examined using UV transillumination.

Semi-quantitative –PCR

Isolation of total RNA

One hundred mg of liver tissue were used for the extraction of RNA by the standard TRIzol® Reagent (Invitrogen™, Carlsbad, CA, USA) according to the manufacturer's procedures and recovered in 100 ml of diethylpyrocarbonate (DEPC)-treated water. In order to remove any possible genomic DNA contamination, the total RNA samples were pretreated using DNA free TM DNase and removal reagents kit (Promega, Co) following the manufacturer's procedures. The integrity and quality of the purified RNA was checked through agarose gel electrophoresis (1%) according to the integrity of 18S and 28S of rRNA bands. The RNA quantity was ascertained Spectrophotometrically (Jenway 6505, UK) as described by Sambrook and Russell [44] with an A260/A280 ratio between 1.7 and 1.9. The purified RNA samples were preserved at ⁻80 °C until use.

Reverse transcription and semi-quantitative polymerase chain reaction (sq-PCR)

Two mg of RNA were reverse transcribed into in a total volume of 20 ml cDNA using the high capacity RNA to PreMix cDNA Kit (iNtRON Biotechnology, Korea). The resulting cDNA was stored at ⁻20 °C for later use or directly used as a semi quantitative PCR template.

Gene expression analysis using semi quantitative PCR

Caspase-3, Bcl-2 and Bax expression were determined in liver using semi quantitative PCR. Oligonucleotide PCR primer pairs were developed for caspase-3, Bcl-2 and Bax genes based on the published primer sequences. The Specificity of the amplification products was confirmed by size estimation on a 1.2% (w/v) agarose gel. GAPDH amplification was used as the housekeeping gene in semi quantitative PCR analysis. In a final volume of 20 ml and 1 ml (0.05 mg) of cDNA was amplified using 2 ml of dNTPs (2.5 mM each), 2 ml of PCR Buffer (10 X), 0.5 ml TaqTM DNA polymerase (5u/ml), 1 ml forward primer (10 pmoles), 1 ml reverse primer (10 pmoles) and 12.5 ml sterilized distilled water. The thermal cycling parameters were: initial denaturation at 94 ^{o}C for 5 min, 30 cycles of amplification at 94^{o}C for 60 s for DNA denaturation, annealing at 52-58^{o}C for 30 s, extension at 72^{o}C for 1 min and a final extension at 72^{o}C for 5 min (Table 1).

Agarose gel electrophoresis

All PCR products were electrophoresed on 2% agarose, stained with ethidium bromide and visualized by UV transilluminator.

Semi-quantitative determination of PCR products

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal control and amplified using its Specific primer. The ethidium bromide-stained gel bands were scanned and the intensity of each band was quantified by densitometry using an image analysis program (IP Lab Gel). The relative abundances for mRNA were estimated as the ratio between the intensity of each mRNA-gene (Caspase-3, Bcl-2 and Bax) and GAPDH gene.

Table 1: Primer sequences and expected product sizes for the genes amplified.

Cdna	Forward primer .(5' -3')	Reverse primer (5' -3')	PCR Product size bp	Reference
Caspase-3	AAATTCAAGGGACGGGTCAT	ATTGACACAATACACGGGATCTGT	05 bp	Liu et al [5]
Bax	AGGATGATTGCTGATGTGGATAC	CACAAAGATGGTCACTGTCTGC	300 bp	Van Der Hoeven et al [46]
Bcl-2	GCTACGAGTGGGATACTGGAGA	AGTCATCCACAGAGCGATGTT	446 bp	Schoemaker et al [47]
GAPDH	CAAGGTCATCCATGACAACTTTG	GTCCACCACCCTGTTGCTGTAG	496 bp	Wiame et al [48]

Histological and Histochemical Investigation

Liver Specimens were fixed in 10% buffered formalin and processed routinely by embedding in paraffin; sections (5 µm) of liver were stained by hematoxylin and eosin for histopathological examination [49] and feulgen technique for DNA[50]. The Feulgen stain reaction specifically stains nuclear DNA with a purple color. DNA analysis was performed by quantitative analysis using Image Pro Plus image analysis software (Media Cybernetics Inc. 2002). Estimation of the optical density relative to DNA contents was performed, and the intensity of the color is directly proportional to the DNA content within the nucleus of the cell.

Statistical Analysis

All the data were analyzed by analysis of variance (ANOVA) by using SPSS 16 Window version. The differences among testing mean values in different groups was done by Duncan's multiple comparison tests and $P \leq 0.001$ values were considered as highly significant and $P \leq 0.05$ was considered statistically significant.

Results

The total phenoicl and flavonoids content

The total phenolic and total flavonoids content of *CH* and *SP* extracts was represented in Table 2. It was noticed that SP has high total phenolic content (50.2 ± 7.18 mg GA eq /g extract) than CH (39.4 ± 0.81 mg GA eq /g extract). While, CH has higher flavonoids content (24.8 ± 3.7 mg Qu eq /g extract) than SP (11.5 ± 0.75 mg Qu eq /g extract).

Table 2: Total phenolic and flavonoids content in *Chlorella vulgaris* and *Spirullina platensis* extracts

Extract	Total Phenolic (mg GA eq /g extract)	Total Flavonoids (mg Qu eq /g extract)
Chlorella vulgaris	39.4 ± 0.81^{b}	24.8 ± 3.70^{a}
Spirulina platensis	50.2 ± 7.18^{a}	11.5 ± 0.75^{b}

Data are represented by the mean ±SE, n = 3, Mean values in the same column bearing the same superscript do not differ significantly (P≤0.05).

Lipid peroxide, glutathione reduced and total antioxidant capacity

The result of lipid peroxidation, glutathione reduced and total antioxidant capacity was illustrated in Table 3. The results show that MSG significantly increased LPO level (p≤0.01) than control groups. In contrast, administration of *CH* and *SP* aqueous extracts in concisions with MSG diminished the content of LPO than that of MSG alone and it is clear that *SP* reduced the LPO to control level. Whereas, significant reduction in reduced GSH was observed in MSG group. In contrast, *CH* and *SP* elevate the reduced GSH than in MSG group. In addition, MSG diminished the total antioxidant capacity than control, while, *CH* and *SP* elevate it than in MSG group.

Table 3: Effect of Chlorella vulgaris, Spirullina platensis aqueous extracts and MSG on liver antioxidant parameters of treated mice

Treatment	Lipid peroxidation (µmol/g)	Glutathione reduced (mg/g tissue)	Total antioxidant capacity (mmol/g)
Control	11.07 ± 0.10^{d}	$24.98+0.60^{c}$	$5.61+0.04^{b}$
CH	9.14 ± 0.11^{e}	$26.40+0.20^{b}$	$4.47+0.15^{d}$
SP	7.56 ± 0.08^{f}	$29.10+0.22^{a}$	$5.63+0.05^{b}$
MSG	21.97 ± 0.48^{a}	$9.22+0.16^{g}$	$1.43+0.01^{f}$
MSG+CH	13.41 ± 0.04^{b}	$15.60+0.13^{f}$	$3.99+0.09^{e}$
MSG+SP	10.59 ± 0.18^{d}	$20.73+0.44^{e}$	$5.12+0.07^{c}$

Data are presented as mean triplicates ± SE Statistically significant $P \leq 0.05$ Groups have the same superscript letter in each parameter indicates statistically insignificant difference between them**.**

DNA Damage evaluation by micronucleus assay

The results obtained are illustrated in Table 4. *CH* and *SP* aqueous extracts did not show significant differences in the average of MNPCEs compared to control. MSG treatment significantly increased the average number of MNPCEs and decreased the PCE/NCE ratio ($p \leq 0.01$) as compared to control groups. Meanwhile, co-administration of either *CH* or *SP* revealed significant decrease in the average of MNPCEs and raise the PCE/NCE ratio ($p \leq 0.01$) than those of MSG.

Table 4: Averages of normo and polychromatic erythrocytes, micronuclei induction and PCE/NCE ratio in bone marrow cells of mice treated with MSG, *CH* and *SP*.

Treatment	Norm chromatic erythrocytes	Polychromatic erythrocytes	MNPCEs	PCE/NCE %
Control	110.20±0.583 [d]	889.80±0.583 [b]	7.20±0.374 [d]	8.07±0.047 [c]
CH	104.40±0.510 [e]	895.60±0.510 [a]	6.60±0.510 [d]	8.57±0.046 [b]
Sp	102.80±0.663 [e]	897.20±0.663 [a]	6.00±0.316 [d]	8.72±0.139 [a]
MSG	186.60±1.03 [a]	813.40±1.03 [e]	34.20±0.374 [a]	4.35±0.029 [f]
MSG + CH	131.80±0.583 [b]	868.20±0.583 [d]	18.20±0.374 [b]	6.58±0.033 [e]
MSG + SP	123.60±0.510 [c]	876.40±0.510 [c]	14.40±0.510 [c]	7.09±0.033 [d]

Data are represented by the mean ±SE, n =8 Mean values in the same column bearing the same superscript do not differ significantly ($P \leq 0.05$).

Apoptosis analysis

Determination apoptosis By Dual AO/EB fluorescent staining

Apoptosis was evaluated via AO/EB staining of bone marrow erythrocytes of treated mice. A non-apoptotic cell will have a bright green appearance and apoptotic cell will have orange to red nucleus as show in Figure 1. There was no difference in the average of apoptotic cells between groups of *CH* and *SP* and control group. Whereas, MSG treatment increased the percentage of apoptotic cells (32.00±1.00) than in control group (14.20±0.374). Whereas, *CH* and *SP* aqueous extracts significantly decreased the apoptotic cells average (24.80±1.985 & 21.00±0.894) compared to MSG group (32.00±1.00) Figure 2.

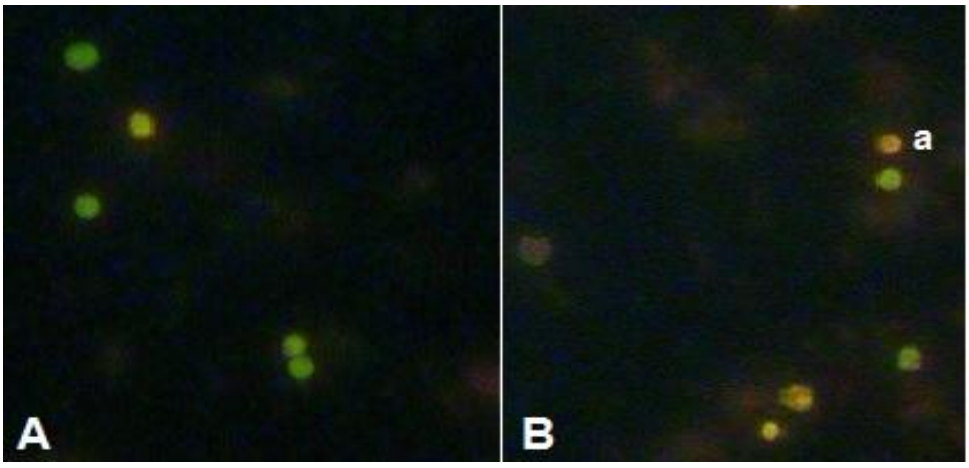

Fig. 1: photomicrograph of bone marrow cells stain with AO/EB stain showing A): Control cells stain with green and B: MSG treated cells showing apoptotic cells with orange-red color (a) and green normal cells.

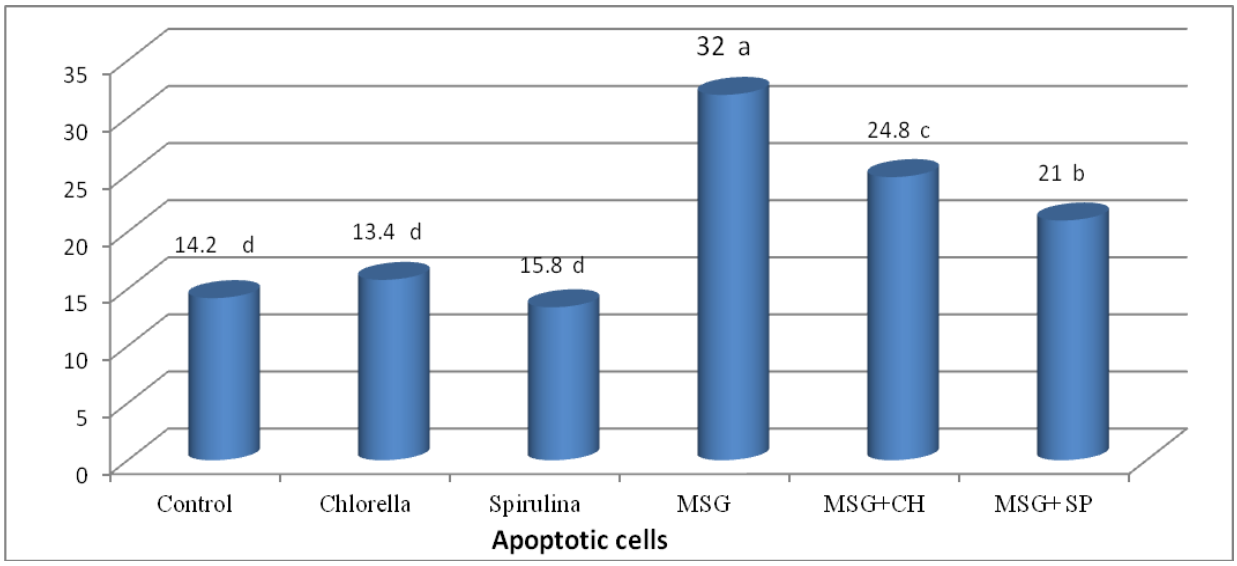

Fig 2: Average of apoptotic cells in bone marrow of mice treated with *CH*, *SP* aqueous extracts and MSG.

DNA Fragmentation

The results point to those cells of control animals did not induce any DNA fragmentation Figure 3. While, characteristic DNA fragmentation in response to monosodium glutamate treatment in hepatocytes was detected by gel electrophoresis as DNA ladder representing a chain of fragments (Figure 3: Lanes 1). Co-administration of both *CH* and *SP* aqueous extracts with MSG inhibit the DNA fragmentation (figure 3: lanes 1±2 &1±3), respectively.

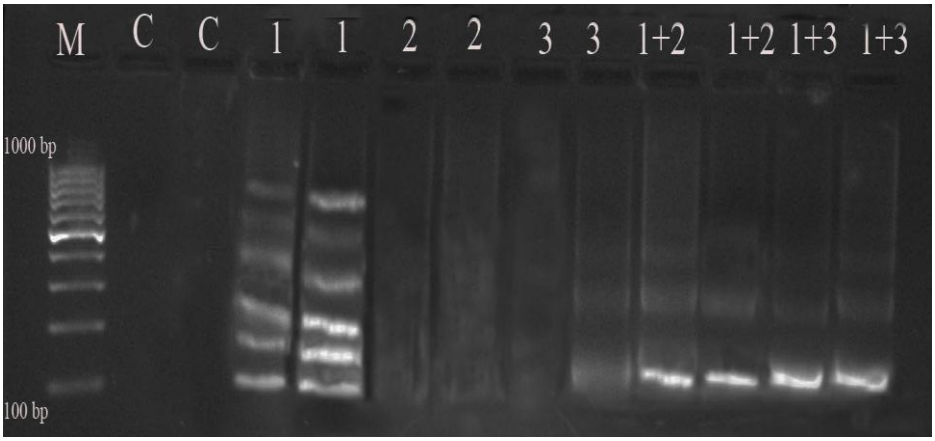

Fig 3: Agarose gel electrophoreses showing DNA fragmentation in liver cells of mice: Lane M: DNA molecular weight marker Lanes 1: MSG treated groups, Lanes 2: *CH* treated group, Lanes 3: *SP* treated group, Lanes 1+2: MSG+*CH* treated group and lane: 1+3 MSG +*SP* treated groups.

Gene expression

Effect of MSG on Caspase-3, Bcl-2 and Bax levels

The changes in mRNA expression of proapoptotic Bax, antiapoptotic Bcl-2 and caspase-3 in mice hepatocytes of all experimental groups compared to the control and to the housekeeping glyceraldehyde-3-phoSphate dehydrogenase gene (GAPDH) are represented in Figure 4-6.

No significant difference was noticed between the control and the groups treated with either SP or CH alone. Animals treated with MSG alone showed a significant increase in the expression of caspase-3 and Bax mRNA. Effect of either *CH* or *SP* aqueous extracts in MSG treated mice accompanied with a significant decline in the expression of Bax Figure, 4 or Caspase-3 Figure, 6. Meanwhile, Bcl-2 expression Figure, 5 showed a significantly decrease in MSG group than control. Either *CH* or *SP* resulted in remarkable changes in the genes

transcription since the expressions of Bax and caspase-3 were significantly decreased while the Bcl-2 expression was significantly increased. However, either *CH* or *SP* aqueous extracts able to restore the Bax/Bcl-2 ratio to the control value although these doses reversed the expression of caspase-3, Bcl-2 and Bax and improved the Bax/Bcl-2 ratio.

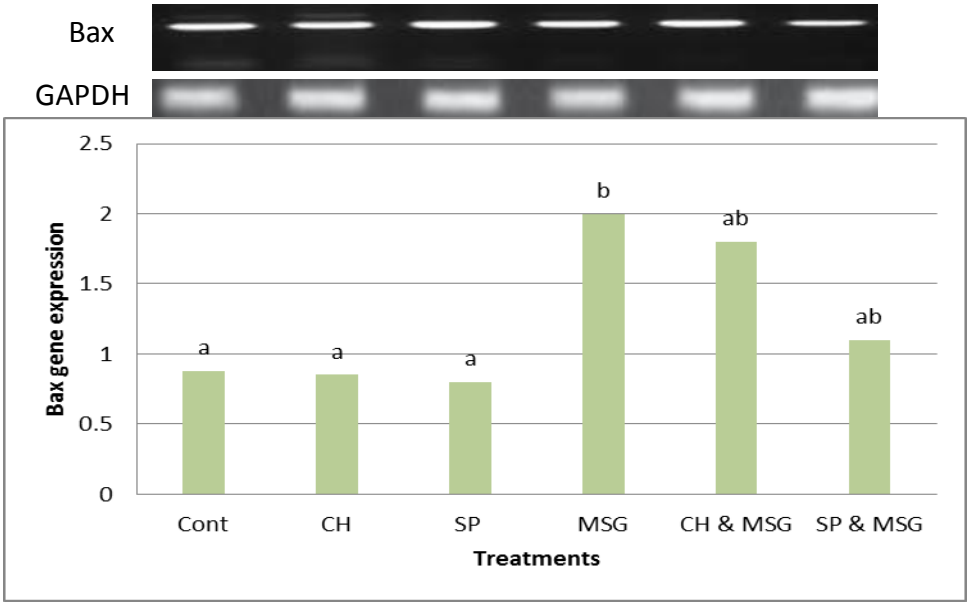

Fig.4: Effect of either *CH* or *SP* aqueous extract on Bax gene expression level in liver of mice induced by MSG. The results illustrated are normalized to the level of GAPDH level and the data are the mean of intensity for each gene divided by that for GAPDH

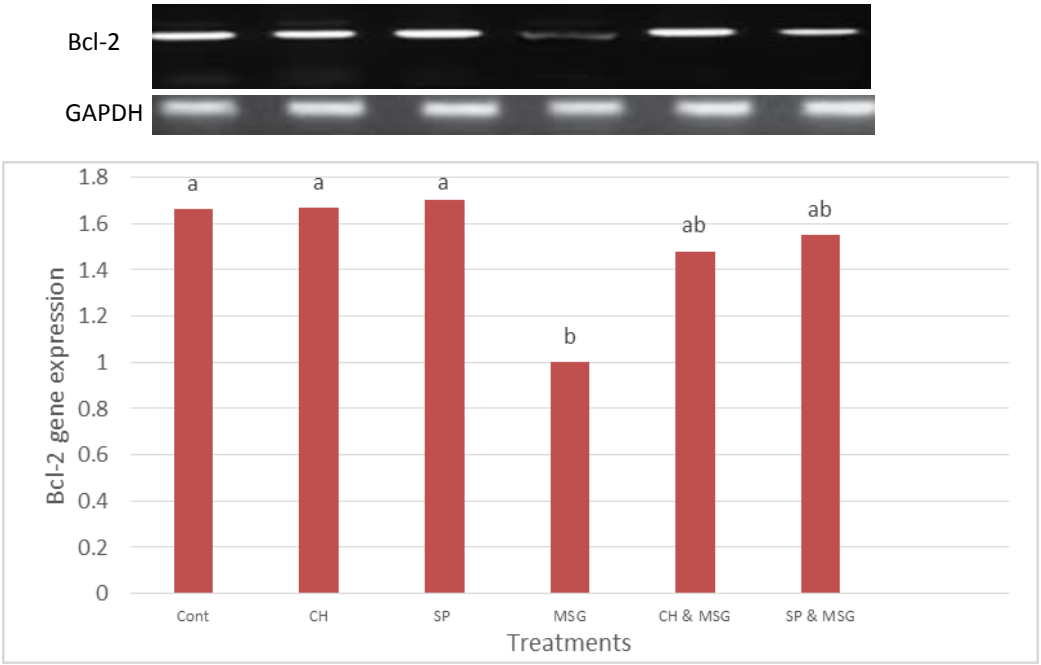

Fig. 5: Effect of either *CH or SP* aqueous extract on Bcl-2 gene expression level in liver of mice induced by MSG. The results illustrated are normalized to the level of GAPDH level and the data are the mean of intensity for each gene divided by that for GAPDH.

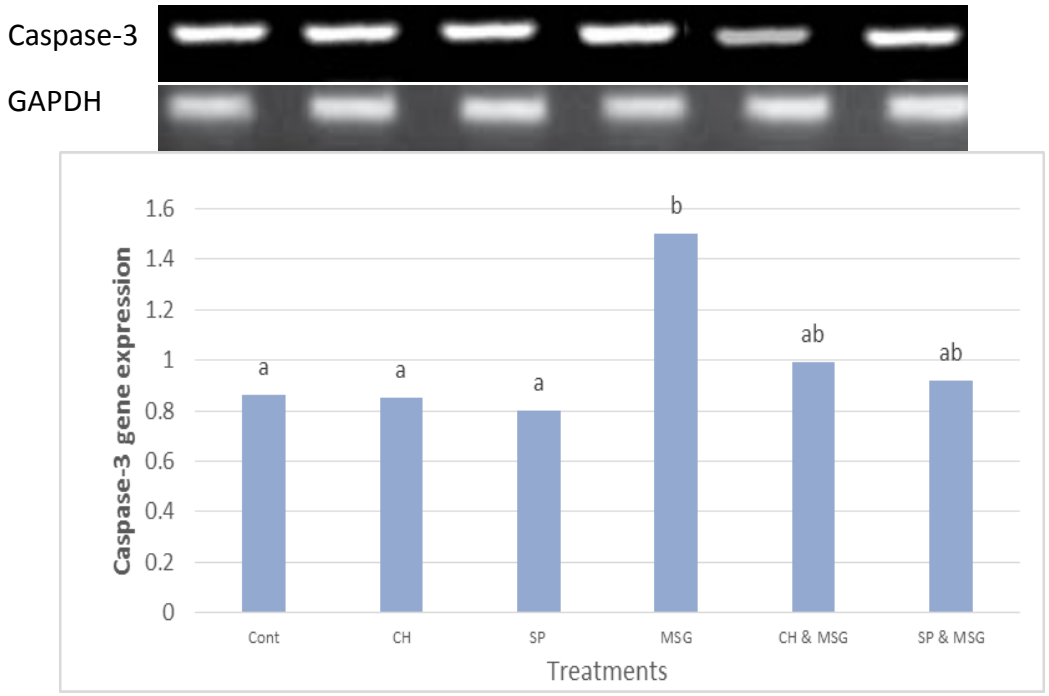

Fig. 6: Effect of either *CH* or *SP* aqueous extract on Caspase-3 gene expression level in liver of mice induced by MSG. The results illustrated are normalized to the level of GAPDH level and the data are the mean of intensity for each gene divided by that for GAPD.

Histological Results

Microscopically examination of liver sections of control mice revealed normal histological architectures as show in Figure, 7**.** No pathological changes could be noticed in hepatocytes of *CH* and *SP* aqueous extracts treated mice. The livers of monosodium glutamate (MSG) treated mice showed sever histological changes. These changes include marked damage in hepatic cells, dilatation and congestion of central vein and sever vacuolar degeneration in most cells with ill-defined outline. The nuclei appear forms of degeneration (pyknosis, karyolysis nuclei in the greater part of hepatocytes Figure, 8. While treatment with *CH* aqueous extract simultaneously with MSG showed some improvement of hepatic cells, most of cells restored their normal configuration, congestion of blood in vein and sinusoids were restricted, nuclei were vesicular and healthy. While, some of cells still suffered from cytoplasm degeneration Figure, 9. Moreover, the treatment of *SP* extract concurrently with MSG revealed more improvements in hepatic tissue and appeared normal histological architecture. Only some dilatation in central vein and sinusoid, little of cellular infiltration were still present Figure 10**.**

Histochemical Results

DNA content was histochemically demonstrated using Feulgen reaction technique Figure, 11A-D, hepatocytes with different features of injured and dying cells were observed in the liver of MSG treated mice Figure, 11B. Such features were not observed in control Figure, 11C or either CH or SP and MSG treated mice liver Figure, 11C&D. Image analyzer measured the mean value for nuclear DNA content per nucleus of different experimental as show in Table 5. There was no difference in liver DNA content between both *CH* and *SP* and the control. MSG caused a significant elevation in liver DNA content. While, either *CH* or *SP* administration parallel with MSG for four weeks showed decrease in DNA content.

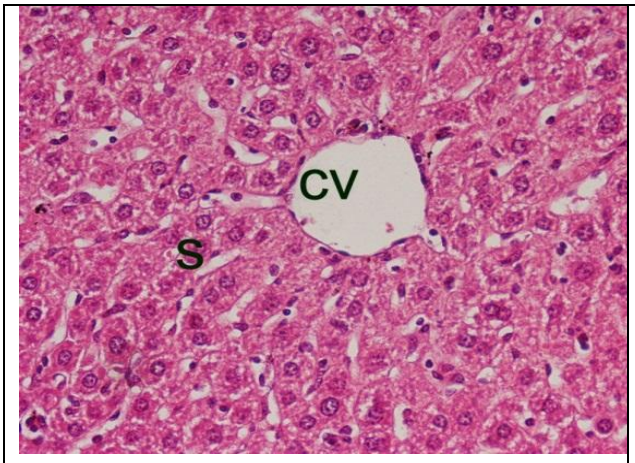

Fig. 7: Section of control liver showing normal structure of hepatic lobule, including central vein(CV), radiating arrangement of hepatic cords, blood sinusoid(s) in between and kupffer cells (H&E x400)

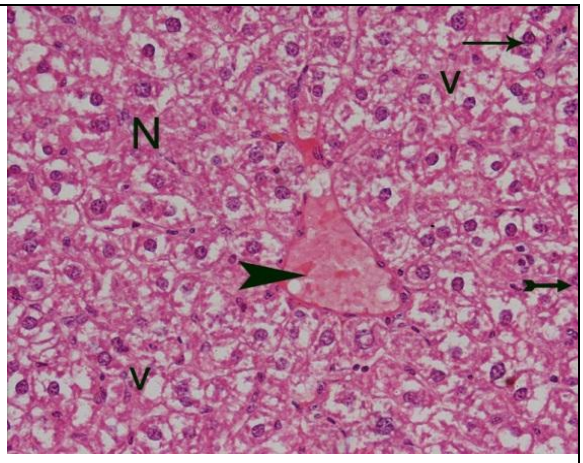

Fig. 8: Section of liver treated with MSG showing marked damage in hepatic cells, dilatation and congestion of central vain (arrow head) , sever vacuolar degeneration (V) with ill-defined outline. While, the nuclei appear shrinkage and small as well as necrosis (N) (pyknosis,(thin arrow) karyolysis nuclei in the majority of hepatocyts. HX&E x400)

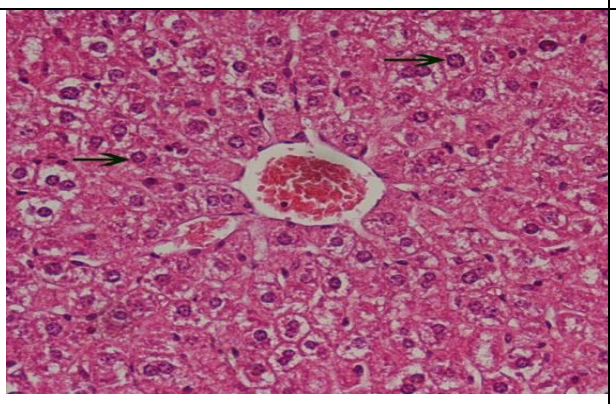

Fig 9: Section of liver treated with MSG plus *CH* showing some improvements .while, congestion in blood vein and sinusoids were restricted, the central nuclei more healthy (arrow) but the degeneration of cytoplasm were still present. (HX & E x400).

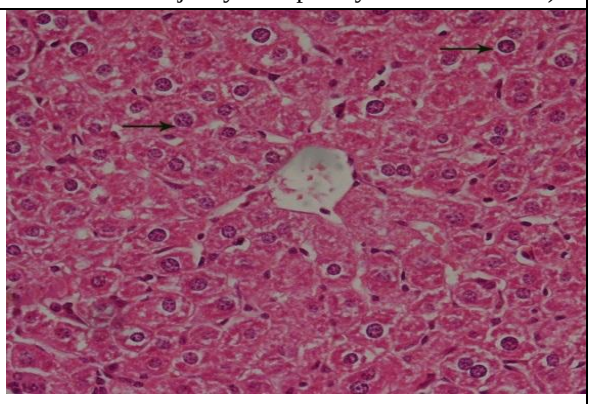

Fig 10: Section of liver treated with MSG plus *SP* showing more improvements in hepatic tissue and appeared normal histological architecture. Only some dilatation in central vein, minute of vacuoles and little of cellular infiltration (HX & E x400) .

Table 5: Optical density of DNA in the liver of mice treated with MSG alone and plus *CH* or *SP*.

	Control	***CH***	***SP***	**MSG**	**MSG + *CH***	**MSG +*SP***
DNA Content	0.115+0.002 ^{c}	0.121+0.002 ^{bc}	0.120+0.020 ^{bc}	0.132+0.030 ^{a}	0.124+0.001 ^{b}	0.123+0.002 ^{b}

Data are represented by the mean ±SE, within raw, means superscript with different letters are significantly different (P≤0.05)

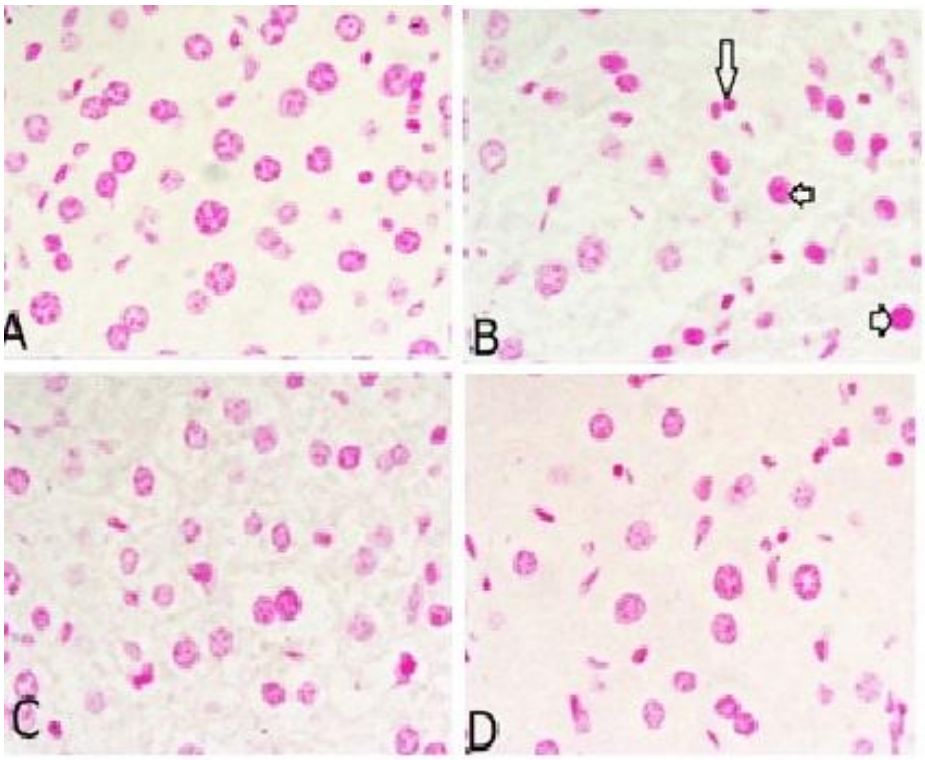

Fig. 11: Sections of the liver of mice in: (A) control group showed normal DNA content; (B) mice treated with MSG showed increase in DNA content; pyknosis (arrow), hyperchromatic nuclei (short arrow); (C, D) mice treated with *CH* or *SP* plus MSG showed more or less normal DNA content (Feulgen stain x400).

Discussion

The interest in marine algal products with their possible promotional health effects increases in regions where algae are considered as rather exotic food. Increased interest about algae as a plentiful source of many nutrients and dietary fiber from the scientific approaches to discover new nutraceuticals and pharmaceuticals, is based on the existence of many bioactive compounds extracted from algal matter [51]. According to several reports, *SP* and *CH* aqueous extracts contain very powerful naturally occurring antioxidant and free radical scavenging activities [30, 20].

In the current research, we estimated the protective role of either *SP* or *CH* extracts in mice by examining their effects on oxidative stress, genotoxicity and cell death pathway induced by MSG. MSG generates ROS causes oxidative DNA damage that may take part in its carcinogenicity[52]. Our study revealed oral intake of MSG has been recommended to enhance the oxidative stress by elevating the malondialdehyde representing lipid peroxidation, decreasing the GSH and total antioxidant activity in liver tissue. These results agreed with results of some previous studies, which suggested that MSG oxidative stress induced by altering the free radical initiating and scavenging enzymes activities [11, 53, 54]. Where free radicals known to attack the cell membrane unsaturated fatty acids and induce lipid peroxidation that is the reason in many oxidative stress pathological events[55]. Results confirmed that administration of *CH* and *SP* reduced the lipid peroxidation and glutathione concentration and total antioxidant activity was elevated. These results were in consistent with Abdel-Daim *et al* [56] which found that *SP* reduced the injury biomarkers of hepatic and renal tissues. The protective effects of *CH* and *SP* were owed to their content of antioxidant active constituents such as C-phycocyanins, carotene, vitamins and minerals reported in SP [57]. Many previous literatures showed that the pretreatment with *SP* might play a vital role in reducing the toxicity of drugs and chemicals and its antioxidant properties show protective effect indicated by the decline of MDA and the elevation of GSH in liver tissue.[58, 59] The potential mechanism that may have led to the increased levels of GSH after *SP* supplementation is the increased content of Vit. C and E in *SP* [60]. In addition, Blas-Valdivia et al [61] demonstrated that *CH* prevents HgCl2-oxidative stress and

cellular damage in the kidney. They anticipated that *CH* carotenes preventing HgCl2-caused lipid peroxidation due to its free radical scavenging property.

In the present work, MSG was able to induce genotoxicity and cytotoxicity. The results illustrated that MSG induced significant increase in DNA damage and cytotoxicity that were supported with the reduction in PCE/NCE, apoptosis induction and DNA fragmentation in hepatocytes. Obtained results are in agreement with Farombi and Onyema[7] they found that the toxicity of MSG was caused mainly by generation of ROS and resulting oxidative stress. Overfeeding of MSG raises obstruction of free radical flow and enhances degenerative diseases[62.] The release of ROS induced DNA fragmentation, apoptosis, increased cytochrome c release from mitochondria to cytosol, down regulated anti-apoptotic Bcl-2 and other mediators; up regulated pro-apoptotic markers[63]. Previous study of Schelman et al [64] indicated that glutamate-induced cell death might be the result of apoptosis and necrosis. In addition, Pavlovic *et al*[65] findings confirmed that MSG resulted in concentration-dependent significantly increased apoptosis. The findings of the present study illustrated that *CH* and *SP* had been show to modulate MSG-DNA damage and apoptosis in bone marrow cells. This result was in agreed with Makpol *et al* [66] they confirmed that *CH* modulated the DNA damage induced by H2O2 and telomere shortening of human fibroblasts derived from different aged individuals. The protective nature of *CH* may be attributing to its high antioxidants and various phenolics that prove to be a potent combination to prevent damage from occurring to DNA. In addition, Saberbaghi *et al* [67] showed that *CH* is capable to postpone cellular ageing by diminishing DNA damage and apoptosis and promote cell cycle progression. It is due to its antioxidant activities, which quenched the reactive ROS and free radicals from attacking the DNA.

In contrast, results presented in our study demonstrated that MSG animal administration induced up-regulation of caspase-3 and Bax mRNA expression and down-regulation of Bcl-2 mRNA expression with resulting change the expression ratio of Bcl-2/Bax mRNA in mice hepatocyte. Previous studies support our findings that treatment with MSG induced thymocytes apoptosis and down regulation protein expression *in vivo*[54, 8]. *In vitro* conditions suggesting that, the Bcl-2/Bax ratio rather than the Bax level is the main determinant for the stimulation of cell death in thymocytes by MSG[68]. In addition, great degrees of changes recorded in our results are in accordance to the histological studies that passed on the liver of MSG treated animals. These changes include marked damage in hepatic tissue and pyknosis, karyolysis nuclei in the majority of hepatocytes. These alterations observed were in agreement with the findings recorded in of Bhattacharya *et al* [69] studied the long-term effect of MSG in mice liver following neonatal exposure. The MSG deformation of the cyto-architecture of the liver could be related to functional changes that may be unsafe to the health of rats. Where, the hepatocytes proliferating cells distortion and dilatation of their central vein highly affected the liver hematopoietic function[70, 71]. Moreover, DNA content appeared increase and atypical hyperchromatin was detected in hepatic cells of MSG treated mice. This result was in consent with Khalaf and Arafat [72] they observed that ultrastructural examination of the thyroid follicles of MSG treated rats revealed follicular cells with short or lost microvilli and hyperchromatic nuclei or pyknotic nuclei with irregular nuclear membrane. Afeefy *et al* [73] previously described a similar ultrastructure in proximal convoluted renal tubules of MSG treated rats.

Moreover, our study confirms the hepatoprotective property of *SP* and *CH* aqueous extracts that represented by remarkable improvement in the transcription of Bax and caspase-3 and Bcl-2 genes. These results correlate with increased in mice cell viability after *SP* or *CH* treatment, suggesting that *SP* or *CH* prevent MSG-induced apoptosis by increasing the Bcl-2 mRNA expression. These observations are in accordance with the findings that algae prevent apoptosis by up-regulating the Bcl-2 protein expression, whereas, intracellular located Bcl-2 is essential to provide antioxidant protection against apoptosis by prevent the liberate of the redox compound of cytochrome c from mitochondria[74]. Thus, Bcl-2 over expression may permit cells to cope better with ROS by allowing increases in endogenous antioxidant enzymes that reverse the ROS-induced decline in Bcl-2 and prevent the cellular death[75]. These results were in agreement with Ismail *et al* [59] who illustrated that *SP* inhibited the rats liver toxicity and carcinogenesis. It has been suggested that the ability of *SP* to inhibit carcinogenesis owing to its antioxidant activities that guard tissues from injury. The

prospective hepatoprotective function of *SP* may be ally with its antioxidant constituents such as selenium, chlorophyll, carotene, tocopherol, phenolic compounds content, vitamin E, and C working individually or in synergy[76].

Conclusion

It might be concluded that *SP* and *CH* aqueous extracts possess hepatoprotective property against MSG and enhanced the activities of liver function, as evidenced by the improvement of MDA, antioxidant activities, histological and histochemical changes and DNA fragmentation. In addition, they showed a potential protection against MSG-induced cytotoxicity, genotoxicity and decreased the expressions of pro-apoptotic Bax, caspase-3 and decrease anti-apoptotic Bcl-2. The *CH* and *SP* extracts mode of action might be performed throughout the prevention or scavenging of ROS. Therefore, these extracts have protective efficiency due to their high content of phenolic and flavonoid compounds and could be considered as an accessible resource of natural antioxidants supplements.

References

1. Wilson R.S., Evans D.A., Bienias J.L., Mendes de Leon C.F., Schneider J.A., Bennett D.A., Proneness to psychological distress and risk of Alzheimer's disease. Neurology, 2003, 61,11,1579–1585.
2. Yuan A.o., Chen G., Zhou Z., Bonney G., Rotimi C., Gene Copy Number Analysis for Family Data Using Semiparametric Copula Model. Bioinformatics and Biology Insights, 2008, 2, 343–355.
3. Peter M.E., Programmed cell death: apoptosis meets necrosis. Nature, 2011, 471, 310–312.
4. Nayanatara A.K., Vinodini N.A., Damodar G., Ahemed B., Ramaswamy C.R., Shabarianth A.M., Ramesh B.M., Role of ascorbic acid in monosodium glutamate mediated effect on testicular weight, Sperm morphology and Sperm count in rat testis. J Chin Clin Med., 2008,3,1–5.
5. Mozes S., Sefcikova Z., Lenhardt L., Racek L. Obesity and changes of alkaline phoSphatase activity in the small intestine of 40- and 80-day-old rats subjected to early postnatal overfeeding or monosodium glutamate. Physiol Res., 2004, 53,177–186.
6. Diniz Y.S., Fernandes A.A., Campos K.E., Mani F., Ribas B.O., Novelli E.L. Toxicity of hypercaloric diet and monosodium glutamate: oxidative stress and metabolic shifting in hepatic tissue. Food Chem Toxicol., 2004, 42,2, 313–9.
7. Farombi E.O., Onyema O.O., Monosodium glutamate-induced oxidative damage and genotoxicity in the rat: modulatory role of vitamin C, vitamin E and quercetin. Hum Exp Toxicol., 2006, 25, 5, 251–9.
8. Pavlovic V., Pavlovic D., Kocic G., Sokolovic D., Sarac M., Jovic Z., Ascorbic acid modulates monosodium glutamate induced cytotoxicity in rat thymus. Bratisl Lek Listy, 2009, 110,4, 205–9.
9. Ahluwalia P., Tewari K., Choudhary P., Studies on the effects of monosodium glutamate MSG on oxidative stress in erythrocytes of adult male mice . Toxicol Lett., 1996, 84,3,161- 5.
10. Manivasagam T. and Subramaniam P., Monosodium glutamate affects the temporal characteristics of biochemical variables in Wistar rats. Pol. J. Pharmacol., 2004, 56,1, 79-84.
11. Singh K. and Pusha A., Alteration in some antioxidant enzymes in cardiac tissue upon monosodium glutamate (MSG) administration to adult male mice. Ind. J. Clin. Bioch., 2005, 20,1,43-46.
12. Pavlovic V. and Cekic S., The effect of monosodium glutamate on rat thymocyte proliferation and BCL-2/ bax protein expression. Arch. Med. Sci., 2006, 2,4, 247-251.
13. El Makawy A. and Abdou H. Modulatory effect of ascorbic acid against food additive monosodium glutamate genotoxicity in rats. J. Genetic Eng. and Biotechnol., 2005, 3,2, 229-253.
14. Yuan Y.V., Walsh N.A., Antioxidant and antiproliferative activities of extracts from a variety of edible seaweeds. Food and Chemical Toxicology, 2006, 44,1144–1150.
15. Mimouni V., Ulmann L., Pasquet V., The potential of microalgae for the production of bioactive molecules of pharmaceutical interest. Curr. Pharm. Biotechnol., 2012, 13, 2733–2750.
16. Herrero M., Alvarez P.J.M., Senorans F.J., Cifuentes A., Ibanez E., Optimization of accelerated solvent extraction of antioxidants from *Spirulina platenses* microalgae. Food Chem., 2005,93, 417-423.
17. Nakashima Y., Ohsawa I., Konishi F., "Preventive effects of Chlorella on cognitive decline in age-dependent dementia model mice". Neuroscience Letters, 2009, 464,3, 193–198.
18. Uchikawa T., Kumamoto Y., Maruyama I., Kumamoto S., Ando Y., Yasutake A., "The enhanced elimination of tissue methylmercury in Parachlorella beijerinckii-fed mice". The Journal of Toxicological Sciences, 2011, 36,1,121–126.

19. Miyazawa T., Nakagawa K., Kimura F., "Chlorella is an effective dietary source of lutein for human erythrocytes". Journal of Oleo Science, 2013,62,10, 773–779.
20. Jiang H., Chen W., Qu L., "ELISA for aging biomarkers induced by telomere dysfunction in human plasma". Journal of Biomedicine and Biotechnology, 2010, ID121947,1-4.
21. Wang H.M., Pan J.L., Chen C.Y., "Identification of anti lung cancer extract from *Chlorella vulgaris* C-C by antioxidant property using supercritical carbon dioxide extraction,". Process Biochemistry, 2010,45,12,1865–1872.
22. Kim K.W., Bai S.C., Koo J.W., Wang X., Kim S.K., Effects of dietary chlorella ellipsoidea supplementation on growth, blood characteristics, and whole-body composition in *Juvenile japanese* flounder paralichthys olivaceus. J. World Aquacult. Soc., 2002, 33, 425–431.
23. Suarez E.R., Kralovec J.A., Noseda M.D., Ewart H.S., Barrow C.J., Lumsdena M.D., Grindley T.B., Isolation, characterization and structural determination of a unique type of Arabino galactan from an immunostimulatory extract of *Chlorella pyrenoidosa*. Carbohydr. Res., 2005, 340, 1489–1498.
24. Cherng, J.Y., Liu C.C., Shen C.R., Lin H.H., Shih M.F., Beneficial effects of Chlorella-11 peptide on blocking LPS-induced macrophage activation and alleviating thermal injury-induced inflammation in rats. Int. J. Immunopathol. Pharmacol., 2010, 23, 811–820.
25. Cherng J.Y. and Shih M.F., Preventing dyslipidemia by *chlorella pyrenoidosa* in rats and hamsters after chronic high fat diet treatment. Life Sci., 2005, 76, 3001–3013.
26. Cherng J.Y. and Shih M.F., Improving glycogenesis in Streptozocin (STZ) diabetic mice after administration of green algae chlorella. Life Sci., 2006, 78, 1181–1186.
27. Gouveia L., Batista A.P., Miranda A., Empis J., Raymundo A., *Chlorella vulgaris* biomass used as coloring source in traditional butter cookies. Innovat Food Sci Emerg Tech., 2007, 8, 433-436.
28. Beheshtipour H., Mortazavian A.M., Haratian P., Effects of *Chlorella vulgaris* and *ArthroSpira platensis* addition on viability of probiotic bacteria in yogurt and its biochemical properties. Eur Food Res Technol., 2012, 235,4,719–728.
29. Banji D., Banji O.J., Pratusha N.G., Investigation on the role of *Spirulina platensis* in ameliorating behavioural changes, thyroid dysfunction and oxidative stress in off Spring of pregnant rats exposed to fluoride. Food Chem., 2013,140,1–2, 321–331.
30. Hosseini S.M., Khosravi-Darani K., Mozafari M.R., Nutritional and medical applications of Spirulina microalgae. Mini Rev Med Chem., 2013,13,1231-7.
31. Basha O.M., Hafez R.A., El-Ayouty Y.M., Mahrous K.F., Bareedy M.H., C-Phycocyanin inhibits cell proliferation and may induce apoptosis in human HepG2 cells. Egypt J. Immunol., 2008,15, 161–167.
32. Avdagic N., Cosovic E., Nakas-Icindic E., Mornjakovic Z., Zaciragic A., *Spirulina platensis* protects against renal injury in rats with gentamicininduced acute tubular necrosis. Bosn J. Basic Med. Sci., 2008, 8, 331–336.
33. Simsek N., Karadeniz A., Kalkan Y., Keles O.N., Unal B., *Spirulina platensis* feeding inhibited the anemia- and leucopenia-induced lead and cadmium in rats. J Hazard Mater., 2009, 164, 1304–1309.
34. Lu J., Ren D.F., Wang J.Z., Sanada H., Egashira Y., Protection by dietary Spirulina platensis against D-galactosamine–and acetaminophen-induced liver injuries. Br J Nutr., 2010,103, 1573–1576.
35. Abdelkhalek N.K., Ghazy E.W., Abdel-Daim M.M., Pharmacodynamic interaction of *Spirulina platensis* and deltamethrin in freshwater fish Nile tilapia, Oreochromis niloticus: impact on lipid peroxidation and oxidative stress. Environ Sci Pollut Res Int., 2015 , 22,4, 3023–3031.
36. Marinova D., Ribarova F., Atanassova, M., Total phenolics and total flavonoids in Bulgarian fruits and vegetables. J Univ Chem Technol Metall., 2005, 40, 255-260.
37. Bruce R.D., An up-and-down procedure for acute toxicity testing. Fundamental and Applied Toxicology.,1985, 5, 151-157.
38. Ohkawa H., Ohishi N., Yagi K., Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem., 1979, 95, 351- 358.
39. Beutler E., Duron O., Kelly B.M., Improved method for the determination of blood glutathione. J Lab Clin Med., 61,882–890.
40. Koracevic D., Koracevic G., Djordjevic V., Andrejevic S., Cosic V., Method for the measurement of antioxidant activity in human fluids.J. Clin. Pathol., 2001,54, 356-361.
41. Krishna G. and Hayashi M., In vivo rodent micronucleus assay: Protocol, conduct and data interpretation. Mut. Res., 2000,455,155-166.
42. Liu K., Liu B., Liu R., Wu X., Dual AO/EB Staining to Detect Apoptosis in Osteosarcoma Cells Compared with Flow Cytometry. Med Sci Monit Basic Res., 2015, 21, 15–20.

43. Perandones C.E., lllera, A.V., Peckham D., Stunzl L.L., Ashman R.F., Regulation of apoptosis in vitro in mature murine spleen T-cell." J. Immunol., 1993,151, 3521-3529.
44. Sambrook J. and Russell D., Molecular Cloning: a Laboratory Manual, 3rd edn. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory., 2001 .
45. Liu W., Wang G., Yakovlev A.G., Identification and functional analysis of the rat caspase-3 gene promoter. J. Biol. Chem., 2002, 277,10, 8273-8278.
46. Van Der Hoeven J.A.B., Moshage H., Schuurs T., Nijboer M., Van Schilfgaarde R., Ploegr R.J., Brain death induces apoptosis in donor liver of the rat., Journal Article, Research Support, Non-U.S. Gov't., 2003,76,8,1150-1154.
47. Schoemaker M.H., Ros J.E., Homan M., Trautwein C., Liston P., Poelstra K., van Goor H., Jansen P.L.M., Han Moshage Cytokine regulation of pro- and anti-apoptotic genes in rat hepatocytes: NF-kB-regulated inhibitor of apoptosis protein 2 (cIAP2) prevents apoptosis. Journal of Hepatology, 2002, 36, 742–750.
48. Wiame I., Remy S., Swennen R., S_agi L., Irreversible heat inactivation of DNaseI without RNA degradation. BioTechniques, 2000, 29, 252-256.
49. Banchroft J., Stevens A., Turner D., Theory and Practice of Histological Techniques.1996, Fourth Ed. Churchil Livingstone, NewYork, London,San Francisco, Tokyo.
50. Feulgen R. and Rosenbeck H.C., Manual of Histological Demonstration Technique. Butter worth & Co (publishers) Ltd. London, Therford, havrhill., 1942.
51. Misurcova L.1., Skrovankova S., Samek D., Ambrozova J., Machu L., Health benefits of algal polysaccharides in human nutrition. Adv Food Nutr Res., 2012, 66, 75-145.
52. Thomas M., Sujatha K.S., George S., Protective effect of *Piper longum* Linn. on monosodium glutamate induced oxidative stress in rats. Indian J Exp Biol., 2009, 47,3,186–92.
53. Paul M.V., Abhilash M., Varghese M.V., Alex M., Nair R.H., Protective effects of alpha-tocopherol against oxidative stress related to nephrotoxicity by monosodium glutamate in rats. Toxicol Mech Methods., 2012,22,8,625–30.
54. Pavlovic V., Cekic S., Kocic G., Sokolovic D., zivkovic V., Effect of monosodium glutamate on Apoptosis and Bcl-2/Bax Protein Level in Rat Thymocyte Culture Physiol. Res., 2007a, 56, 619-626.
55. Lobo V., Patil A., Phatak A., Chandra N., Free radicals, antioxidants and functional foods: Impact on human health. Pharmacogn Rev., 2010, 4,8,118-26.
56. Abdel-Daim M.M., Abuzead S.M.M., Halawa S.M., Protective Role of *Spirulina platensis* against Acute Deltamethrin-Induced Toxicity in Rats. PLoS ONE, 2013,8,9, e72991.
57. Upasani C.D., Balaraman R., Protective effect of Spirulina on lead induced deleterious changes in the lipid peroxidation and endogenous antioxidants in rats. Phytother. Res., 2003, 17, 330-334.
58. Karadeniz A., Cemek M., Simssek N., The effects of *Panax ginseng* and *Spirulina platensis* on hepatotoxicity induced bycadmium in rats. Ecotoxicol Environ Saf., 2009,72,1, 231–5.
59. Ismail F. M., Ali A.D., Fernando A., Abdraboh E.M., Gaur L.R., Ibrahim M.W., Raj G.H.M., Ouhtit A., Chemoprevention of rat liver toxicity and carcinogenesis by Spirulina, Int J Biol Sci., 2009, 5,4, 377–387.
60. Wu G., Fang Y.Z., Yang S., Lupton J.R., Turner N.D., Glutathione metabolism and its implications for health. J Nutr., 2004,134,3,489–492.
61. Blas-Valdivia V., Ortiz-Butron R., Pineda-Reynoso M., Hernandez-Garcia A., CanoEuropa E., *Chlorella vulgaris* administration prevents HgCl2-caused oxidative stress and cellular damage in the kidney. Journal of Applied Phycology ., 2011,23, 1, 53-58.
62. Diniz, L.E.C., Ruas C.F. Carvalho V.P., Torres F.M., Ruas E.A., Santos M.O., Sera T., Ruas P.M., Genetic Diversity Among Forty Coffee Varieties Assessed by RAPD Markers Associated with Restriction Digestion, Brazilian Archives of Biology and Technology, 2005, 48,4, 511-521.
63. Banu S.K., Stanley J.A., Lee J., Stephen S.D., Arosh J.A., Hoyer P.B., Burghardt R.C., Hexavalent chromium-induced apoptosis of granulosa cells involves selective sub-cellular translocation of Bcl-2 members, ERK1/2 and p53. Toxicol Appl Pharmacol., 2011, 251, 253–266.
64. Schelman W.R., Andres R.D., Sipe K.J., Kang E., Weyhenmeyer J.A., Glutamate mediates cell death and increases the Bax to Bcl-2 ratio in a differentiated neuronal cell line. Brain Res Mol Brain Res., 2005,128, 160-169.
65. Pavlovic V., Cekic S., Sokolovic D., Modulatory effect of monosodium glutamate on rat thymocyte proliferation and apoptosis. Brat Lek Listy, 2006,107,185–191.

66. Makpol S., Yaacob N., Zainuddin A., Yusof M.A.Y., Ngah W.Z. W., *chlorella vulgaris* modulates hydrogen peroxide-induced dna damage and telomere shortening of human fibroblasts derived from different aged individuals. Afr. J. Trad. CAM., 2009, 6,4, 560 – 572.
67. Saberbaghi T., Abbasian F., Yusof M.A.Y., Makpol S., Modulation of Cell Cycle Profile by *Chlorella vulgaris* Prevents Replicative Senescence of Human Diploid FibroblastsEvid Based Complement Alternat Med., 2013, article ID 780504,1-12.
68. Pavlovic V., Pavlovic D., Kocic G., Sokolovic D., Jevtovic-Stoimenov T, Cekic S, Velickovic D., Effect of monosodium glutamate on oxidative stress and apoptosis in rat thymus. Mol Cell Biochem., 2007b ,303, 1-2, 161-6.
69. Bhattacharya T., Bhakta A., Ghosh S.K., Long term effect of monosodium glutamate in liver of albino mice after neo-natal exposure. Nepal Med Coll J ., 2011; 13,1,11-16.
70. Eweka A.O., Om'Iniabohs F.A.E., Histological studies of the effects of monosodium glutamate on the small intestine of adult Wistar rat. Electron J Biomed., 2007, 2,14–18.
71. Eweka A.O., Om'Iniabohs F.A.E., Histological studies of the effects of monosodium glutamate on the Liver of adult Wistar rats. The Internet Journal of Gastroenterology., 2008, 6 available.
72. Khalaf H. A., and Arafat E.A. , Effect of different doses of monosodium glutamate on the thyroid follicular cells of adult male albino rats: a histological study. Int J Clin Exp Pathol ., 2015,8,12, 15498-15510.
73. Afeefy A.A., Mahmoud M.S., Arafa M.A., Effect of Honey on Monosodium Glutamate Induced Nephrotoxicity (Histological and Electron Microscopic Studies) Journal of American Science. 2012,8,146–156.
74. Saitoh T., Nakayama M., Nakano H., Yagita H., Yamamoto N., Yamaoka S., TWEAK induces NF-kappaB2 p100 processing and long lasting NF-kappaB activation. J Biol Chem., 2003,278, 36005–36012.
75. Hildeman D.A., Mitchell T., Kappler J., Marrack P ., T cell apoptosis and reactive oxygen species. J Clin Investig., 2003,1, 575-581.
76. Garcia Martinez D., Rupérez F.J., Ugarte P. Tocopherol fate in plasma and liver of streptozotocin-treated rats that orally received antioxidants and Spirulina extracts. Int J Vitam Nutr Res., 2007, 77, 263–271.