ISSN: 2230-9926

Available online at http://www.journalijdr.com

IJDR

International Journal of Development Research
Vol. 08, Issue, 12, pp.24695-24699, December, 2018

ORIGINAL RESEARCH ARTICLE OPEN ACCESS

PROTECTIVE ROLE OF *CHLORELLA VULGARIS* AND *SPIRULINA PLATENSIS* ON OXIDATIVE STRESS AND TOXICITY INDUCED BY 1,2 DIMETHYLHYDRAZINE IN RATS

[1]Mohy EldinAbd EL-Fattah Abd EL-Atty, [2]Mohamed Mohamed Abdel-Daim, and[3]Basant Salah Eldeen Mohammed

[1]Faculty of Science, Suez Canal University, Egypt
[2]Faculty of Veterinary Medicine, Suez Canal University, Egypt
[3]Faculty of Science, Suez Canal University, Egypt

ARTICLE INFO

Article History:
Received 22nd September, 2018
Received in revised form
16th October, 2018
Accepted 03rd November, 2018
Published online 31st December, 2018

Key Words:
Colon cancer, DMH,
Spirulina platenesis,
Chlorella vulgaris,
Antioxidant biomarker.

ABSTRACT

1, 2-Dimethylhydrazine (DMH), a toxic environmental pollutant is a well establishedprocarcinogen. DMH undergoes metabolism in the liver, resulting in the production of oxidative stress. *Chlorella vulgaris* (CV) and *Spirulinaplatenesis*(SP) are the single-celled microalgae, which have been reported to have antioxidant and anticancer properties. The present study was carried out to investigate the protective roles of CV and SP against DMH-induced oxidative stress in rat liver. Rats were divided into five groups. Group 1 served as -ve control, Group 2 received DMH, and served as +ve control, Group 3 received CV, Group 4 received SP, Group 5 received CV and SP for 5weeks. Groups 3, 4 and 5 were given DMH. Liver function tests, hepatic lipidperoxidation (LPO) and oxidative stress markers were examined by evaluating serum alanineaminotransferase (sALT), serum aspartateaminotransferase (sAST)malondialdehyde (MDA), catalase (CAT), reduced glutathione (GSH) and superoxide dismutase (SOD). In our study, the levels of sAST and sALT significantly increased in +ve control group compared with -ve control group. Enhanced LPO in the liver of +ve control rats were accompanied by a significant decreased in the activities of GSH, SOD and CAT, and increased in MDA. Oral administration of CV and SP to DMH-injected rats significantly reduced sALT, sAST, MDA, and enhanced the activities of GSH, SOD and CAT in the liver. Our study suggested that, CV and SP exert their protective roles against DMH-induced oxidative stress and toxicity in rat liver by modulating the extent of lipid peroxidation and augmenting antioxidant defense system.

Citation: Dr. Mohy Eldin Abd EL-Fattah Abd EL-Atty, Dr. Mohamed Mohamed Abdel-Daim and Basant Salah Eldeen Mohammed. 2018. "Protective role of chlorella vulgaris and spirulina platensis on oxidative stress and toxicity induced by 1, 2 dimethylhydrazine in rats", *International Journal of Development Research*, 8, (12), 24695-24699.

INTRODUCTION

1, 2-Dimethylhydrazinedihydrochloride is a highly toxic and carcinogenic and affects number of body organs including liver. It is metabolized in the liver and produces highly reactive electrophiles i.e., carbonium ions and alkyl free radicals which severely damage the liver leading to necrosis and fatty infiltration(Sharma and Sharma, 2011). *Spirulina*, a planktonic blue green alga , is an old-styled food of some Mexican and African people and is one of the oldest forms of life growing in warm water alkaline volcanic lakes on earth for the last 3.5 billion years or so (Khan *et al*., 2005).

***Corresponding author:** Dr. MohyEldinAbd EL-Fattah Abd EL-Atty
Faculty of Science, Suez Canal University, Egypt

Spirulina has solid antioxidant potential; its true health-protective merit has only recently been uncovered. Phycocyanobilin (PCB), the chromophore bound to chief protein (phycocyanin), can work as a potent inhibitor of NADPH oxidase, the enzyme complex that is considered a leading source of pathological oxidant stress in a wide range of health disorders (McCarty, 2007).*Spirulina* is well-known to have antioxidant features, attributed to molecules such as phycocyanin, β-carotene, tocopherol, g-linolenic acid and phenolic compounds (Chopra and Bishnoi, 2007). For instance, selenium-containing phyco-cyanin from *Spirulina* has long been believed to have strong superoxide and hydrogen peroxide radical scavenging activities (Huang *et al*., 2007). *Chlorella vulgrais*is one of the green eukaryotic, found on earth since the Precambrian period. It is a unicellular

microalga that grows in fresh water and has founded on earth since the pre-Cambrian period 2.5 billion years ago. Since then its genetic integrity has remained constant (Safi *et al*., 2014). The extraction of *Chlorella vulgaris* contains a large amount of dietary antioxidant compounds, including in lutein, α-carotene, β-carotene, ascorbic acid, phenolic compounds and atocopherol (Vijayavel *et al*., 2007). These bioactive compounds are able to scavenge the free radicals; moreover, the antioxidant features, including suppression of hemoglobin-motivated linoleic acid peroxidation, reducing power, ferrous chelating, 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging and scavenging of a superoxide anion radical-generated non-enzymatic system were studied (Kitada *et al*., 2009).

MATERIALS AND METHODS

Chemical: 1, 2-dimethylhydrazine dihydrochloride was purchased from Sigma-Aldrich (St. Louis, Missouri, USA). Pure *SpirulinaPlatensis* and *Chlorella Vulgrasis* were purchased from National Research Center, Cairo, Egypt. All kits of biochemical parameter were purchased from Biodiagnostic CO. (Cairo, Egypt).

Animals and experimental design: Male rats weighing (150g -200g) were supplied by Laboratory Animals Unite (Ismailia, Egypt). For induction of colonic cancer, rat will be injected subcutaneously with DMH (40 mg/kg body weight / twice weekly,) for 5 weeks. Treatment with SP and CV were launched before, during the course of DMH, at dose of 500 mg/kg daily for SP(Abdel-Daim*et al*., 2013; Hidalgo-Lucas *et al*., 2014) and 50 mg/Kg body weight daily for CV. A total of 60 rats were randomly divided into 5 groups, each group started with 12 rats as following:

Group 1: (negative control) animals receivedsubcutaneous injection of normal saline for five weeks.

Group 2: (positive control) animals received subcutaneous injection of DMH for five weeks.

Group 3: DMH+ CV; animals were given CV orally daily for 5 weeks with DMH treatment. CV was given for 7 days before thes.c injection of DMH.

Group 4: DMH+ SP; animals were given SP orally daily for 5 weeks with DMH treatment. Sp was given for 7 days before the s.c injection of DMH.

Group 5: DMH + CV+SP; the animals orally administrated a combined doses of SP + CV daily for 5 weeks with DMH treatment. Sp and CV were given for 7 days before thes.c injection of DMH.

Biochemical assay: The biochemical markers of hepatic damage, including serum (AST, ALT, and ALP), Tissue lipid peroxidation (MDA) and oxidative stress markers were assessed; superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH) were estimated in tissue homogenate based on the protocol of the purchased kit from Biodiagnostic CO. (Cairo, Egypt).

Histopathological examination: liver sections were taken immediately from the colon, fixed in 10% buffered formalin, dehydrated in ethanol (50–100%), cleared in xylene, and embedded in paraffin. Sections (4–5 mm thick) were prepared and then stained with hematoxilin and eosin (H&E).

Statistical analysis: The present results were analyzed using Statistical Package for Social Science (SPSS) version (SPSS Inc., Chicago) for windows. Result were expressed as mean ± SE (n=8). One way ANOVA followed by Duncan test were used for analysis. P value less than 0.05 was considered significant.

RESULTS

Serum biochemical markers of liver functions in male albino rats: After 6 weeks of experiment, the activities of serum transaminases (ALT, AST) and ALP showed significant (P≤0.05%) increase in DMH group compared to control group. While treated groups; *Spirulinaplatenesis, Chlorella vulgaris* and its combination showed significant decrease in ALT, AST and ALP activities compared DMH group (table 1 and Figure 1-3).

2-Hepatic lipid peroxidation and antioxidant status in male albino rats: After 6 weeks of experiment, DMH group elucidated lipid peroxidation of tissue evidenced significant

Table 1. Serum liver enzyme activities and biochemical marker levels in the control and treated groups

Parameter	Experimental groups				
	Control	DMH	DMH-CV	DMH-SP	DMH-CV-SP
AST (U/L)	63.71 ±3.45^{d}	190.92 ±9.14^{a}	104.65±5.76^{b}	82.89±3.77^{c}	70.51±3.87^{cd}
ALT (U/L)	27.10 ±1.43^{d}	108.42±3.15^{a}	66.30±3.88^{b}	42.45±2.90^{c}	30.34±1.81^{d}
ALP (U/L)	68.13 ±2.91^{d}	200.64 ±9.24^{a}	117.29±5.14^{b}	87.32±4.15^{c}	70.29±3.43^{d}

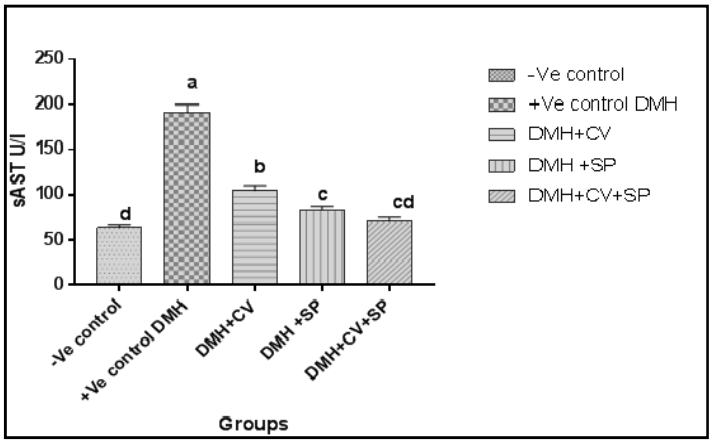

Figure 1. Effect of SP, CV and its combination SP+CV on sAST in DMH induced colon cancer in rats. Data represent the mean value ± S.E from 8 rats/ group

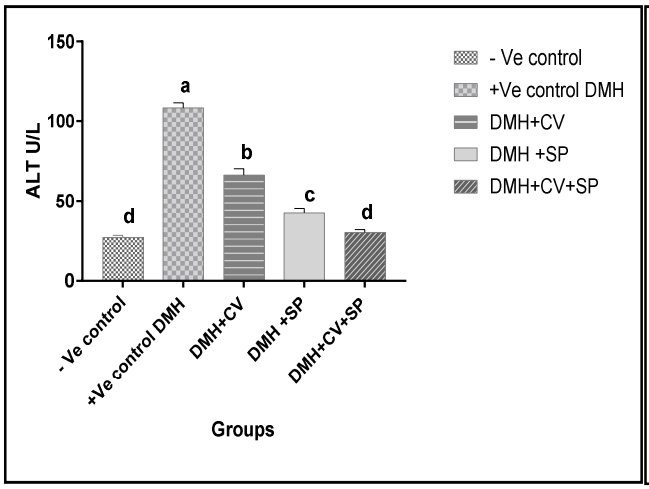

Figure 2. Effect of SP, CV and its combination SP+CV on sALT in DMH induced colon cancer in rats. Data represent the mean value ± S.E from 8 rats/ group

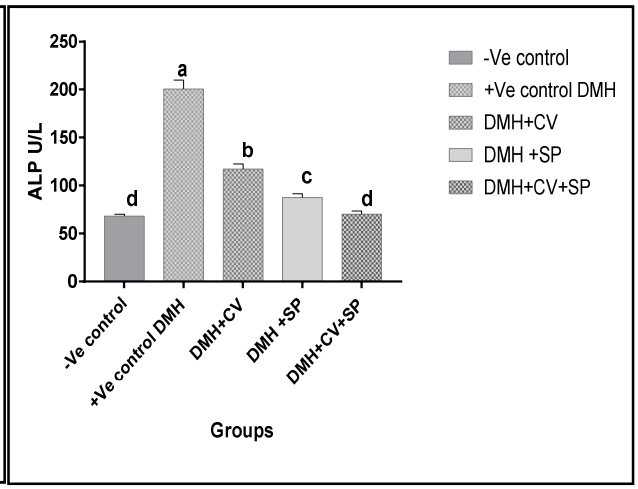

Figure 2. Effect of SP, CV and its combination SP+CV on sALP in DMH induced colon cancer in rats. Data represent the mean value ± S.E from 8 rats/ group

Table 2. Antioxidant enzyme activities and lipid peroxidation level in the liver tissue of control and treated groups

Parameter	Experimental groups				
	Control	DMH	DMH-CV	DMH-SP	DMH-CV-SP
MDA(nmol/g tissue)	43.39 ±2.99^{c}	100.18±3.74^{a}	67.42±3.71^{b}	52.71±3.74^{c}	43.16±2.95^{c}
GSH (mg/g tissue)	69.11 ±3.80^{a}	32.88 ±2.04^{d}	49.49±1.01^{c}	56.64±0.98^{b}	63.87±1.55^{a}
SOD (U/g tissue)	21.07 ±0.63^{a}	5.76 ±0.41^{e}	13.13±0.49^{d}	16.00±0.47^{c}	19.35±0.43^{b}
CAT (U/g tissue)	2.07 ±0.04^{a}	0.50 ±0.03^{d}	1.21±0.05^{c}	1.62±0.05^{b}	1.96±0.07^{a}

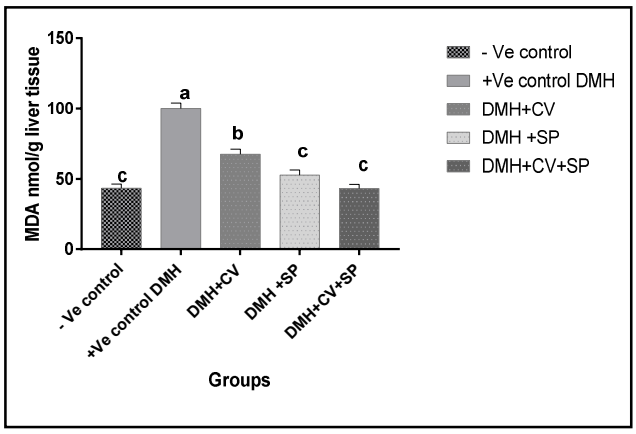

Figure 4. Effect of SP, CV and its combination SP+CV on liver tissue MDA in DMH induced colon cancer in rats. Data represent the mean value ± S.E from 8 rat/ group

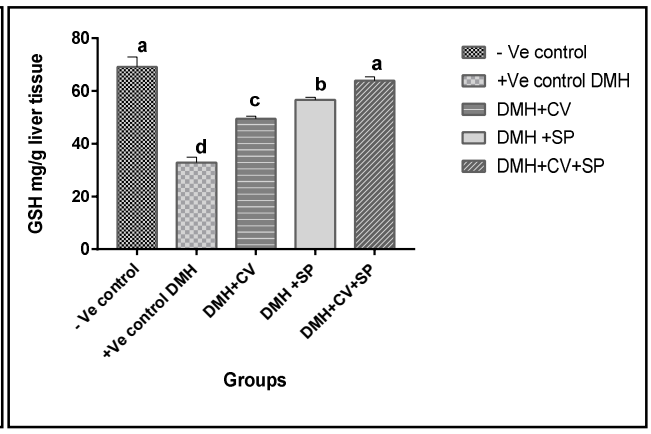

Figure 5. Effect of SP, CV and its combination SP+CV on liver tissue GSH in DMH induced colon cancer in rats.Data represent the mean value ± S.E from 8 rats/ group

(P≤0.05%) high level of MAD compared to control rats. While treated groups; *Spirulinaplatenesis, Chlorella vulgaris* and its combination group showed significant decrease in MDA level compared DMH group. The tissue SOD, CAT activity and GSH level significant (P≤0.05%) decrease in DMH group compared with control group. Whereas *Spirulinaplatenesis, Chlorella Vulgasis* and its combination group elevated SOD, CAT activity and GSH level compared with DMH group, There were significant alteration between each other (Table 2 and Figure 4-7).

Histopathological result: The histopathological change of liver tissues in DMH group and the *Spirulinaplatenesis, Chlorella vulgaris* and its combination compared to that of normal control showed in Figure (8).Control groups showed normal liver tissue. DMH group showed moderated portal inflammation and vascular degeneration.

SP, CV and its combination showing marked improvement in liver near to normal. Data are expressed as mean ± SE (*n* = 8).Means with different superscript letters within the same row differ significantly at P < 0.05. AST (as partate aminotransferase), ALT (alanine aminotransferase), ALP (alkaline phosphatase), DMH (dimethyle hydrazine), DMH+CV (dimethyle hydrazine+ *Chlorella vulgaris*), DMH+SP (dimethyle hydrazine-*Spirulinaplatensis*), DMH+CV+SP (dimethyle hydrazine+ *Chlorella vulgaris*-*Spirulinaplatensis*). Data are expressed as mean ± SE (*n* = 8).Means with different superscript letters within the same row differ significantly at P < 0.05. MPO (Myleoperoxidase), MDA (malondialdehyde), GSH (reduced glutathione), SOD (superoxide dismutase), CAT (catalase), DMH (dimethyle hydrazine), DMH+CV (dimethylehydrazine+*Chlorella vulgaris*), DMH+SP (dimethylehydrazine+*Spirulinaplatensis*), DMH+CV+SP (dimethyle hydrazine+ *Chlorella vulgaris*+*Spirulinaplatensis*).

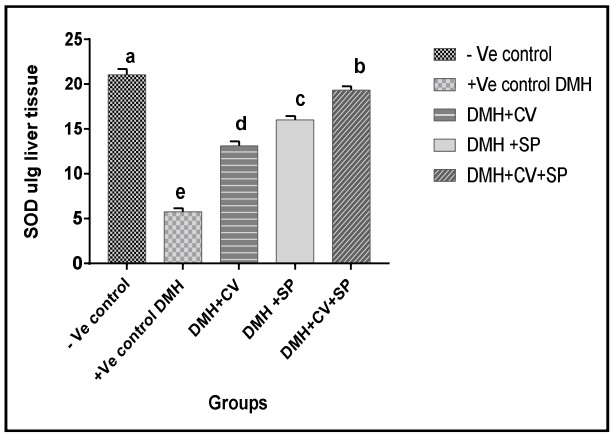

Figure 6. Effect of SP, CV and its combination SP+CV on liver tissue SOD in DMH induced colon cancer in rats. Data represent the mean value ± S.E from 8 rats/ group

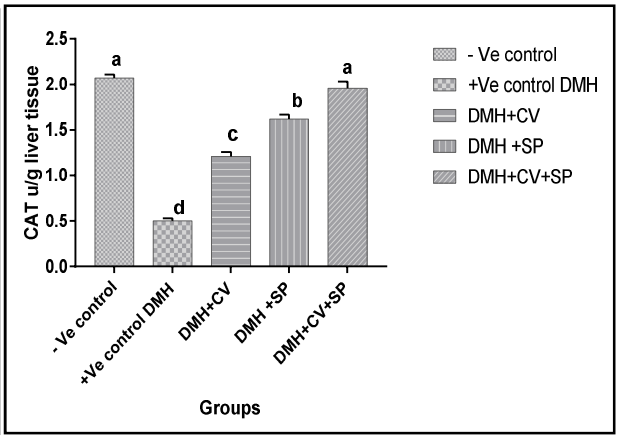

Figure 7. Effect of SP, CV and its combination SP+CV on liver tissue CAT in DMH induced colon cancer in rats. Data represent the mean value ± S.E from 8 rats/ group

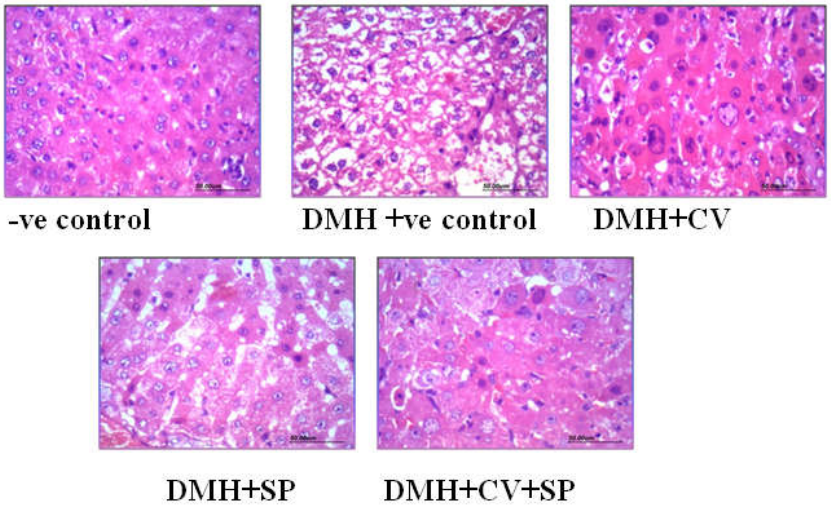

Figure 8.H&E staining for liver tissue in control and DMH- induced colon cancer in rats

DISSCSSION

1, 2 Dimethyl hydrazine (DMH), is a potent colon carcinogen, motivating colorectal tumors in experimental animals and is the most widely adopted model of chemically induced colon carcinogenesis (Hamiza *et al*., 2012).(Fiala, 1975) has proved that active metabolite of DMH is excreted through bile and is responsible for its carcinogenic effect on colon while passing through digestive tract. The activities of liver marker enzymes and their variation reflect the overall Qcolon carcinoma),hiked the level of enzymes in the liver, including in ALT, AST and ALP compared to the control group, and then our treatment with SP, CV and its combination decreased the level of these enzymes in comparison to the DMH group. Alteration the level of liver enzymes is adopted to assess liver function and the elevation in level of these enzymes considered as hepatic lesions or hepatocytes damage (Fatemi *et al*., 2006). Occurrence of hepatocytes necrosis motivated the release of these enzymes in blood circulation; moreover increased levels of AST refer to liver damage in some diseases such as viral hepatitis, cardiac infarction and muscle injuries. While ALT is a liver specific parameter, catalyzing the change of alanine to pyruvate and glutamate, so, it is suitable for the observance of liver damages (Dadrass *et al*., 2014). And unlike ALT, found mainly in the liver, AST is existed in many other tissues, foremost of which are heart, muscle, kidney, and brain. Almost all liver damage cases hike the levels of these two enzymes (ALT and AST) while elevation of serum ALP occurs in disorders of the biliary system mainly. This change in the level of serum liver enzymes could be pertinent to the toxic effect of DMH on liver. Additionally, thesis results have beensupported by histopatholiogical examination performed on the liver. It has shown that antioxidants could inhibit prolifieration of cancer cells (Chinery *et al*., 1998). Oxygen–derived free radicals are probable mediator of such toxicity, and their role in cancer is well recognized (Cerutti, 1994). The biochemical marker estimated MAD, SOD, CAT and GSH are assessed in liver homogenate, as it is revealed the liver enzymes are more sensitive indicator of distal neoplasm than blood and colon tissue. A marked reduction in hepatic GSH and antioxidant enzymes (SOD and CAT) was observed in DMH rats. In contrast, a significant increase in the MDA level was evident compared to the normal control group. Administration of CV and SP produced a marked increase in GSH , improved the activity of all the antioxidant enzymes and reduced MDA relative to the DMH group. In (-ve control group) normal liver tissue, there was preserved architecture with hepatocytes arranged in thin cell plates and separated by sinusoids, portal areas and bile ductules. Hepatocytes have abundant eosinophilicconversion in metabolism that occurred during malignancyand led to organs dysfunction (Rajkapoor *et al*., 2005). Transaminases are considered important class of

enzyme linking carbohydrates and amino acid metabolism and these enzymes and have established a relation between the intermediates of tricarboxylic acid cycle. Increased ALP and AST activities in colon cancer–bearing rats describe the presence of hepaticdamages as a result of cancer cells invasion. The outcomes in this experiment showed that injection of DMH (for motivating cytoplasm and central nucleus. In +ve control (DMH group), liver tissue showed disturbed architecture with hepatocytes arranged in thick cell plates with markedly enlarged dysplastic nuclei with increased nucleocytoplasmic ratio and marked nuclear pleomorphism and hyperchromasia. There were scattered mitotic figures and bizarre giant cells, as well as, moderate portal inflammation and vacuolar degeneration. In DMH+CV group**,** there was mild improvement of liver tissue that restored hepatic architecture; moreover hepatocytes showed central nuclei and other foci showed residual moderate dysplastic changes. In DMH-SP group, there was notable improvement of liver tissue that showed restored hepatic architecture; meanwhile hepatocytes showed central nuclei and other foci showed residual mild dysplastic changes. In DMH+CV+SP group, there was moderate improvement of liver tissue that showed restored hepatic architecture; meanwhile hepatocytes showed central nuclei and other foci showed residual mild dysplastic changes.

Conclusion

Our study suggested that, the hepato-protective effect of CV and SP is attributed to its ability to reduce the rate of lipid peroxidation and to enhance the antioxidant defense status, in the liver induced by DMH. The antioxidant activity also suggests that a dietary supplement of CV and SP may confer a beneficial effect against oxidative stress.

REFERENCES

Abdel-Daim, M.M., Abuzead, S.M. and Halawa, S.M., 2013. Protective role of Spirulinaplatensis against acute deltamethrin-induced toxicity in rats. *PLoS One,* 8(9): e72991.

Cerutti, P.A., 1994. Oxy-radicals and cancer. The Lancet, 344(8926): 862-863.

Chinery, R. *et al*., 1998. Antioxidants reduce cyclooxygenase-2 expression, prostaglandin production, and proliferation in colorectal cancer cells. *Cancer research*, 58(11): 2323-2327.

Chopra, K. and Bishnoi, M., 2007.ntioxidant profile of Spirulina: a blue-greenmicroalga. *Spirulina in Human Nutrition and Health:* 101-118.

Dadrass, M., Nejati, V., Tukmechi, A. and Hobbenaghi, R., 2014.The Protective Effect of Cell Wall and Cytoplasmic Fraction of Selenium Enriched Yeast on 1, 2-Dimethylhydrazine-induced Damage in Liver. Research in Molecular Medicine, 2(1): 39-45.

Fatemi, F. *et al*., 2006. Changes in hepatic cytosolic glutathione S-transferase activity and expression of its class-P during prenatal and postnatal period in rats treated with aflatoxin B1. Archives of toxicology, 80(9): 572-579.

Fiala, E.S., 1975. Investigations into the metabolism and mode of action of the colon carcinogen 1, 2-dimethylhydrazine. Cancer, 36(S6): 2407-2412.

Hamiza, O.O. *et al*., 2012. Amelioration of 1, 2 Dimethylhydrazine (DMH) induced colon oxidative stress, inflammation and tumor promotion response by tannic acid in Wistar rats. Asian Pacific Journal of Cancer Prevention, 13(9): 4393-4402.

Hidalgo-Lucas, S. *et al*., 2014.Benefits of oral and topical administration of ROQUETTE Chlorella sp. on skin inflammation and wound healing in mice. Antiinflamm Antiallergy Agents Med Chem, 13(2): 93-102.

Huang, Z., Guo, B., Wong, R. and Jiang, Y., 2007. Characterization and antioxidant activity of selenium-containing phycocyanin isolated from Spirulinaplatensis. Food Chemistry, 100(3): 1137-1143.

Khan, Z., Bhadouria, P. and Bisen, P., 2005.Nutritional and therapeutic potential of Spirulina. Current pharmaceutical biotechnology, 6(5): 373-379.

Kitada, K. *et al*., 2009. Antioxidant and antibacterial activity of nutraceutical compounds from Chlorella vulgaris extracted in hydrothermal condition. Separation Science and Technology, 44(5): 1228-1239.

McCarty, M.F., 2007. ‘‘Iatrogenic Gilbert syndrome’’–A strategy for reducing vascular and cancer risk by increasing plasma unconjugated bilirubin. Medical hypotheses, 69(5): 974-994.

Rajkapoor, B., Murugesh, N., Chodon, D. and Sakthisekaran, D., 2005. Chemo-prevention of N-nitrosodiethylamine induced phenobarbitol promoted liver tumors in rat by extract of Indigoferaaspalathoides. *Biological and Pharmaceutical Bulletin,* 28(2): 364-366.

Safi, C., Zebib, B., Merah, O., Pontalier, P.-Y.andVaca-Garcia, C., 2014. Morphology, composition, production, processing and applications of Chlorella vulgaris: a review. Renewable and Sustainable Energy Reviews, 35: 265-278.

Sharma, A. and Sharma, K.K., 2011. Chemoprotective role of triphala against 1, 2-dimethylhydrazine dihydrochloride induced carcinogenic damage to mouse liver. Indian Journal of Clinical Biochemistry, 26(3): 290-295.

Vijayavel, K., Anbuselvam, C. and Balasubramanian, M., 2007.Antioxidant effect of the marine algae Chlorella vulgaris against naphthalene-induced oxidative stress in the albino rats. Molecular and cellular biochemistry, 303(1-2): 39-44.

****