Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Vol 13 [4] March 2024: 177-184

Online ISSN 2277-1808
Journal's URL: http://www.bepls.com
CODEN: BEPLAD

ORIGINAL ARTICLE **OPEN ACCESS**

The Chemoprevention of *Arthrospira platensis* against Diethyl nitrosamine induced Hepatocellular Carcinoma via Amelioration of Oxidative Stress in Mice

Neelam*, Priyanka Gupta and Versha Sharma
Osteo immunology research lab, Department of Zoology, School of Biological Sciences, Dr. Harisingh Gour Vishwavidyalaya, Sagar, M.P. 470003, India
Corresponding mail id- neelam981@gmail.com

ABSTRACT
Considering that natural antioxidant products do not have any negative side effects; they are an important component in the treatment and prevention of cancer. The purpose of the research was to explore whether Arthrospira platensis (SP) has hepatoprotective characteristics against hepatocellular carcinoma (HCC) in mice under the experimental conditions. The research was carried out with a group of fifty male Balb/c mice, they were then divided into five unique groups. Following is a list of the groups: (I) Group1: the normal control group, (II) Group 2: Hepatocellular carcinoma (HCC) group that was induced by a single injection of Diethyl nitrosamine (DEN) at a dosage of 100 mg/kg intraperitoneally (I. P). This was followed by injections of carbon tetrachloride (CCl4) twice a week 0.5 mg/kg body weight intraperitonially for a total of 22 weeks, beginning 14 days after the initial injection. (III) Group 3: Oral administration of SP at a dose of 250 mg/kg was given to the HCC group. (IV) group 4, an oral dosage of SP containing 500 mg/kg was given to the animals, whereas (V) group 5, a dosage of 250 mg/kg of SP was given and considered as treatment control. The data revealed that the treatment of spirulina resulted in a significant decrease in the activity of glutamate oxaloacetate transaminase (GOT) also known to be AST, glutamate pyruvate transaminase (GPT) also known to be ALT, gamma-glutamate transaminase, and malondialdehyde (MDA). In addition, there was a notable rise in the total protein level, as well as in the activity of superoxide dismutase (SOD) and catalase (CAT), which were both much higher than normal levels. Spirulina has been shown to exhibit chemoprotective qualities, which may be linked to its antioxidant activity and its capacity to reduce lipid peroxidation has been demonstrated in every single study that has been conducted. In addition to this, it has been shown that spirulina can enhance the levels of pro-inflammatory cytokines, which in turn can significantly reduce inflammation.
Keywords*: HCC; inflammatory mediators; spirulina; antioxidant; oxidative stress*

Received 11.12.2023 Revised 20.02.2024 Accepted 06.03.2024

INTRODUCTION

Chronic liver disease (CLD) is a widespread disorder worldwide all over the world and is marked by a series of adverse effects, beginning with steatosis and culminating in hepatocellular carcinoma (HCC) [1]. Liver carcinoma that is primary Hepatocellular carcinoma, often known as HCC, is the most common and important type of liver cancer globally [2]. There are several significant risk factors contribute to the development of liver cirrhosis in persons who have hepatocellular carcinoma (HCC). These risk factors include drinking alcohol, being infected with hepatitis C and B viruses [3,] being overweight or obese, having diabetes, and being exposed to water that has been polluted with chemicals [4]. It is difficult to diagnose HCC at an early stage, the prognosis is not positive, and there are no definitive treatment options available [5]. These are the factors that contribute to the risks associated with HCC. In spite of the fact that there are traditional therapies for liver cancer (HCC), for instance chemotherapy, radiation therapy, and surgery, all of which are linked with adverse effects, liver transplantation is regarded to be the most successful treatment. On the other hand, there are considerable obstacles to overcome, such as the restricted supply of liver organs and the possibility of tumour recurrence following transplant procedure [6]. Therefore, it is of the utmost importance to make progress in the development of anticancer chemicals that are both more productive and less toxic, such as natural compounds, in order for prevention or delay the emergence of liver cancer. Diethyl nitrosamine, often known as DEN, is a carcinogen that is found in high concentrations in a variety of environmental goods, including cheese, alcoholic drinks, soybeans, processed meat, pesticides, tobacco products, and cosmetics [7]. There is a strong correlation between the

carcinogenic qualities of DEN and its capacity to create reactive oxygen species (ROS) in excessive amounts. ROS have the potential to cause damage to several biological components, including lipids, DNA, and proteins [8]. There is a large period of time that is required for the use of DEN as the only inducer of liver cancer. As a result, the incorporation of advancing chemicals namely carbon tetrachloride (CCl4) becomes essential for the acceleration of the development of liver cancer [9]. Cellular damage can be caused by CCl4 either by covalently coupling its reactive intermediates to the cellular constituents or also by peroxidizing membrane lipids in conjunction alongside the free radical intermediates, which eventually leads to damage to the intracellular membrane [10]. Furthermore, the p450 enzymes have the ability to enhance the dehalogenation process of CCl4, which ultimately leads to the production of trichloromethyl radicals throughout the process. Following this, these radicals are able to accompanied by the sulfhydryl groups of glutathione and by thiol proteins, which ultimately results in a decrement in the antioxidant defenses [11]. Inflammation of the liver is responsible for the majority of instances of hepatocellular carcinoma (HCC) [12], and there is numerous different molecular mechanism that make happen progression and malignancy of HCC [2]. Species that are capable of interacting with DNA bases are produced as a result of oxidative and nitrative stress as well as lipid peroxidation [13]. This interaction leads to the production of DNA adducts, which have the potential to induce mutations, In a wide variety of malignant circumstances. Oxidative stress takes a significant part in the progression and the emergence of hepatocellular carcinoma (HCC) [14], since it is a contributor to the migration, invasion, and dissemination of HCC [15]. Furthermore, oxidative stress has the ability to cause damage to hepatocytes, which can result in an increase in aberrant polyploidization and the initiation of inflammation [16]. In addition to mixed lesions that are together referred to as steatohepatitis, the outcomes of lipid peroxidation have the potential to activate stellate cells in the liver that are responsible for the production of collagen and to result in the activation of pro-inflammatory cytokines. The degradation and death of hepatocytes, the infiltration of inflammatory cells, and the formation of fibrosis are all examples of these lesions [17]. An example of a filamentous microalga that is classified as a member of the cyanobacterium group is the *Spirulina platensis* (SP) species. When included into a diet, it is recognized for the powerful antioxidant, anti-inflammatory, anti-cancer properties [18]. As a result of its high concentration of protein, carbohydrates, sterols, and polyunsaturated fatty acids, as well as vital minerals as an example zinc, calcium, magnesium, iron, manganese, and selenium, it is utilised as a nutritional supplement for human consumption. Tocopherol, vitamin E, ascorbic acid, and vitamin B 12 are all found naturally in spirulina [19], a natural source of vitamin E. In the past, people from Asia, Mexico, and Africa have used it as a source of sustenance. Due to the substance's potential antioxidant, antibacterial, and antiparasitic properties, it is now the subject of a significant amount of study. The potential uses of this substance in the treatment of an extensive varieties of illness, including allergies, ulcers, anemia, heavy metal toxicity, and radiation exposure, are also being researched [20]. The hepatoprotective effects of spirulina were investigated in this study with the intention of determining whether or not they are effective against hepatocellular carcinoma (HCC) caused by diethyl nitrosamine. This was accomplished by investigating the effect that spirulina has on oxidative stress as well as the production of cytokines that are associated with inflammation.

MATERIAL AND METHODS

Materials

Arthrospira platensis was obtained from (Healthy hey organic, Tamil Nadu, India). Diethyl nitrosamine (DEN), carbon tetrachloride (CCl_4), and other chemicals namely Riboflavin and Thio-barbituric acid were procured from Sigma Aldrich. Additionally, AST and ALT commercial kits were ordered from company Bio diagnostics (Lucknow, India). Total protein colorimetric kits were procured from the Abcam company.

Animals

This study was carried off with the assistance of fifty male Balb/c mice with an average body weight (b.wt.) of 20-25 gm. These mice were obtained from the Department of Zoology at Banaras Hindu University, India. The mice were housed in a plastic cage, for a period of one to two weeks. This allowed them to acclimate to the laboratory environment, which included a temperature range of 22 to 25 degrees Celsius and a 12-hour light and dark photoperiod. Throughout the course of the study, the animals were fed a standard commercial diet and had gain access to water *ad libitum*. Both the Committee for the Supervision of Experiments and Purpose of Control on Animals (CPSCEA) of India and another is the Institutional Animal Ethical Committee (IAEC) in India, led by Dr. Harisingh Gour Vishwavidyalaya, Sagar, (M.P.), gave their approval for the experiment.

Induction of HCC DEN model

A single dosage of DEN (100 mg/kg b.wt.) was jab intraperitoneally (I.P.) to the mice participating in the experiment [21]. Additionally, to induce cancer formation, CCL4 was administered (0.5 ml/kg/I.P.) biweekly, beginning 14 days after the DEN injection and continuing for 22 weeks of experiment [22].

Experimental design

Following the acclimatisation period, mice were arbitrarily allocated into five Groups using a similar method: Group (I): The mice in this group were administered 0.9% normal saline through an oral gavage. Group (II): The mice in this group were treated with DEN/CCl4, as indicated earlier. Group (III) mice were administered a jab of 250 mg/kg of Spirulina [23]. Group (IV) mice were administered a dosage of 500 mg/kg of Spirulina, while Group (V) served as the control group and got a jab of 250 mg/kg of Spirulina [24]. Spirulina treatment was administered daily via stomach tube after the induction of HCC for a duration of 8 weeks.

Biochemical examination

At the conclusion of the 28-week study, biochemical examination was conducted. The mice were rendered unconscious by injecting thiopental sodium into their abdominal cavity at a dosage of 50 mg/kg b.w. [25]. The blood sample for examination was obtained through the retroorbital venous plexus from mice involved in the study. It was thereafter put through centrifugation at a speed of 3000 rpm for an extent of 20 minutes to separate the serum. The serum was subsequently stored at a temperature of -20°C up to the time of the measurement of Serum Glutamate pyruvate transaminase (SGPT), Serum Glutamate oxaloacetate transaminase (SGOT) [26], and total protein [27] using quantitative colorimetric commercial kits.

Anti-oxidant assay

The blood sample was obtained in a tube containing heparin and thereafter put through centrifugation at a speed of 4000 revolutions per minute for an extent of 15 minutes at a temperature of 4 degrees Celsius. This process was carried out to separate the plasma from the erythrocyte lysate. The resulting samples were then maintained at a temperature of -20 degrees Celsius until they were used for the oxidative/antioxidant assay. The activities of Superoxide dismutase (SOD), Lipid peroxidation (LPO), and catalase activity (CAT) were evaluated using the relevant procedures described by placer et al. [26].

Histopathological Examination

The liver tissues sample obtained out of all mice in the experiment were prepared thereafter divided into sections aimed at histopathological investigation. The liver tissue was preserved using 10% neutral formalin, dehydrated using different alcohol concentrations, cleared out with xylene, and finally placed in paraffin blocks for embedding. The sections thereafter prepared using a 6 μm thickness and subsequently stained with hematoxylin and eosin staining (H&E) [28]. The Leica DMRBE light microscope from Japan, equipped with a Leica DFC 295 digital camera and computer, was utilised to capture photographs of the histological alterations in the liver tissue across all experiment groups.

The data statistical analysis

To verify the existence of differences between the various experimental groups, a one-way analysis of variance (ANOVA) was carried through, and a Tukey test was also carried out. This was done with the assistance of GraphPad software Inc. The p-value that is lower than 0.05 is considered as statistically significant. Further the information is provided in the form of the mean value, supplemented or subtracted by the standard error of mean (SEM).

RESULTS

Biochemical markers

Evidence (Shown in Fig 1) reveals a noteworthy rise ($P < 0.001$) in the serum activity of liver function test GPT and GOT in mice that were administered DEN/CCl4 in comparison to the normal control group. The administration of Spirulina, on the other hand, led to a noteworthy decrement ($P < 0.001$) in the levels of GPT and GOT when compared to the HCC group. Spirulina therapy did not result in any discernible differences in enzyme activity among the group that was treated as Spirulina control. On the other hand, when compared to the DEN/CCl4 group, the enzyme activity was dramatically reduced ($P < 0.001$) by the treatment with SP, which brought it very close to the normal level. When the group that was treated with DEN+CCl4 was compared to the normal group, it was observed that there was a substantial decline in the total protein level ($P < 0.001$). Particularly noteworthy is the fact that the SP treatment led to a significant rise ($P < 0.05$) in the total protein level as compared to the experimental group that was subjected to the DEN/CCl4. In point of fact, there was not a significant alteration seen in the enzyme activities among the group that was treated to SP control and the group that served as the normal control.

Antioxidant activity assay

In comparison to the normal group (NC), the malondialdehyde activity shown a significant incline ($P < 0.001$) in the group that was affected by liver cancer. It is quite remarkable that the SP treatment demonstrates a significant decrement ($P < 0.001$) in the MDA activity when compared with the HCC group. On the other hand, as compared to the normal group, the group that was treated with DEN/CCl4 exhibited

a noteworthy reduction ($P < 0.0001$) in antioxidant activity, particularly in SOD and CAT. At the same time, the group that was treated with SP had a noteworthy rise ($P < 0.001$) in the levels of SOD and CAT activity as compared to the group that was treated with HCC (seen in Fig 2). Additionally, the higher dose of Spirulina showed higher activity of antioxidant activity compared to lower dose of Spirulina.

Histopathology

According to the findings of the histological examination, the liver tissue in the normal group exhibited a structure that is normal. In contrast, the liver tissue of mice that were palliated with DEN/CCl4 displayed atypical hepatocellular carcinoma (HCC) lesions. These lesions were distinguished by severe necrosis, polymorphic area, intense inflammation with mononuclear cells infiltration, cytoplasmic inclusion bodies, severe large vacuolar disintegration of liver cells, and pronounced congestion of blood sinusoids. Several beneficial effects on the liver tissue were seen as a result of the treatment of SP. These benefits included a minor enlargement of the sinuses, a reduction in the number of cancer lesions, mild to moderate vascular degeneration, and apoptosis of the hepatocytes (as in Fig 3). In most cases, the histological alterations demonstrated an improvement in the groups who were treated with SP. Additionally, a larger dose of SP led to a better recovery when compared to a lesser dose of Spirulina. The SP control group had a liver architecture that was normal.

DISCUSSION

This study reveals that SP has a chemoprotective impact against liver cancer caused by DEN+CCl4. Currently there are no drugs in the market that can provide complete protection or aid in the regeneration of liver cells [29]. This is despite the fact that there have been major developments in modern medicine. Further, there are some drugs that have the potential to cause unpleasant responses. Therefore, it is of the utmost importance to investigate substitutive natural and biological substances for the therapeutics of liver diseases that have a high effectiveness and a lower threshold of toxicity. Injecting DEN has been shown to result in the generation of free radicals, which alter antioxidant levels and contribute to oxidative stress and carcinogenesis [30]. This has been proven by a number of studies. Damage to the liver comes about as a consequence of the presence of DEN, which leads to an unstable metabolism in the liver and significant changes in the activity of blood enzymes [31]. Both DEN and CCL4 are responsible for the damage that they produce to the liver, which is a consequence of increased lipid peroxidation (LPO) during their metabolic processes and a reduction in the synthesis of antioxidant enzymes [32]. Lipid peroxides have a deleterious effect on the DNA of cells, which can result in genetic alterations and the development of cancer. In addition, the antioxidant provides protection against reactive oxygen species (ROS). Additionally, superoxide dismutase (SOD) has the ability to assist the changeover of two superoxide radicals into hydrogen peroxide (H2O2) and oxygen (O_2) through a process known as disproportionation. Additionally, by providing support for antioxidant enzymes, CAT makes it easier for hydrogen peroxide (H_2O_2) into water (H_2O) [32, 41]. Other symptoms include high levels of SGOT and SGPT [33], as well as various types of tumours [34]. Through histological examination of liver tissue, it has been established beyond a reasonable doubt that DEN+CCl4 has a carcinogenic impact. This study demonstrates the presence of necrotic lesions present in liver cells, that there are varying degrees of steatosis, and numerous vacuoles of diverse sizes present in the hepatocytes [35]. The present investigation revealed that the administration of DEN in conjunction with CCL4 results in a significant increment in the blood activities of GOT, and GPT enzymes, while simultaneously resulting in a decrease in the serum total protein level as compared to mice that were subjected to normal circumstances. These finding indicates that there was damage to the liver (liver injury). Additionally, a significant rise in the amount of MDA is associated with a reduction in the activity of both catalase and superoxide dismutase. Necrosis, widespread vacuolar degeneration of the liver cells, strong inflammation with infiltration of mononuclear cells, and the presence of cytoplasmic inclusion bodies were all seen in the group that was treated with DEN+CCl_4 according to the findings of the histological investigation. According to the findings of our research, the administration of spirulina to animals efficiently improves the liver enzyme biomarkers that are affected by the injection of DEN+CCl_4. Compared to mice who were injected with DEN+ CCl_4, animals that were treated with spirulina had lower levels of liver enzymes (AST, ALT, and ALP) [36]. These finding are consistent with the findings of another study that had shown the similar results. It is possible that the antioxidant property of spirulina, which protects liver tissue from degeneration, is responsible for the augmentation of liver enzyme activity that occurs as a result of therapy with spirulina [37]. When compared to mice that were injected with DEN+CCl4, the current findings indicate that treatment with spirulina showed a large decrease in MDA levels and a considerable increase in antioxidant activity, notably an increase in SOD and CAT [38]. According to a previous study, which also indicated that supplementation with spirulina led to a substantial incline in CAT and SOD activity, as well as a decline in MDA levels [39,40], these findings are consistent with the interpretation of the current investigation [41]. The hepatoprotective efficiency of spirulina was

demonstrated using histological research, which showed that treatment with SP improved hepatic cancer lesions and decreased the number of hepatocytes that died. One possible explanation for this is that spirulina possesses antioxidant properties and has the capacity to reduce oxidative stress. An examination using histological techniques demonstrated that the administration of spirulina resulted in an improvement of liver tissue.

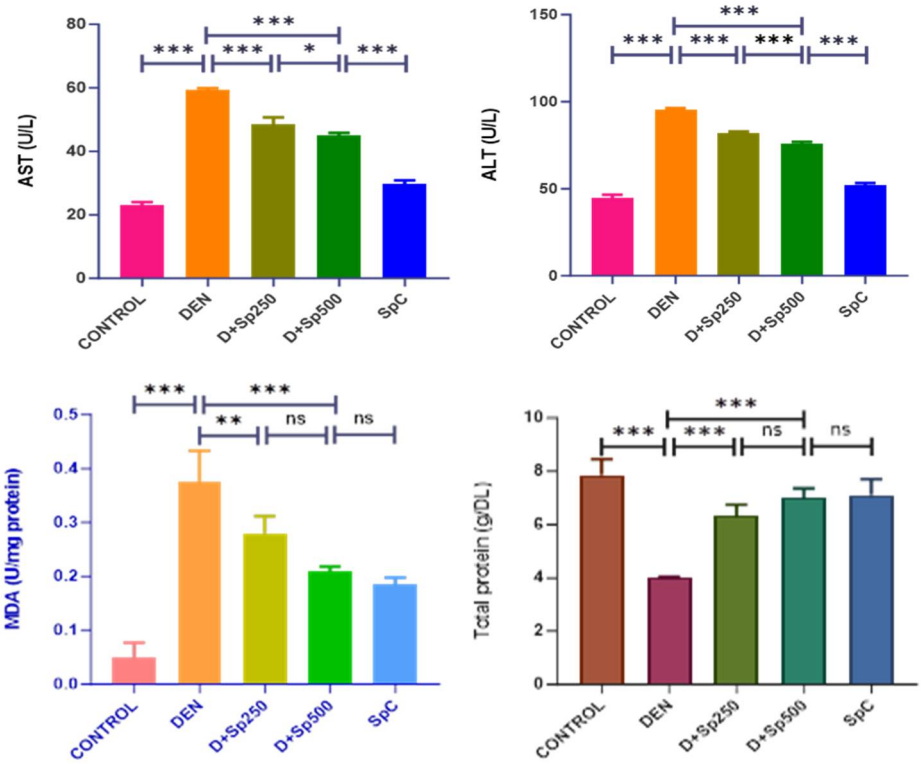

Fig 1. The expression of ALT, AST enzyme level, MDA and total protein was analyzed. The results were evaluated by using 1-way ANOVA Analysis was performed using GraphPad prism software 10.0. Values are reported as mean ± SEM (n = 7) and similar results were obtained in two independent experiments. Statistical significance was defined as *P<0.05, **P<0.01, ***P<0.001, with respect to Control vs DEN+CCl_4, DEN+CCl4 vs D+Sp250, DEN+CCl_4 vs D+Sp500, D+Sp250 vs D+Sp500, D+Sp500 vs SpC group.

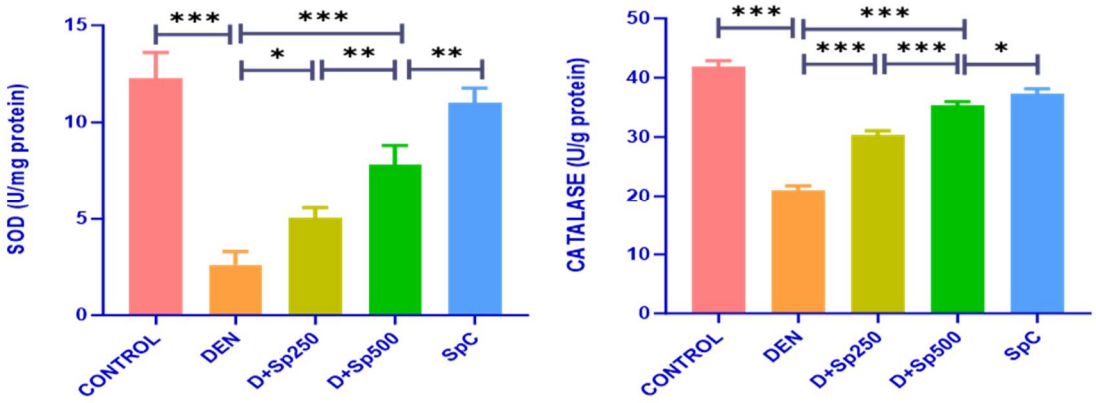

Fig 2. The antioxidant marker enzymes such as Superoxide dismutase (SOD), Catalase (CAT), and stress marker Lipid peroxidation were tested in the all the experimental group animals. The results were evaluated by using 1-way ANOVA Analysis was performed using GraphPad prism software 10.0. Values are reported as mean ± SEM (n = 7) and similar results were obtained in two independent experiments. Statistical significance was defined as *P<0.05, **P<0.01, ***P<0.001, with respect to Control vs DEN+CCl_4, DEN+CCl_4 vs D+Sp250, DEN+CCl4 vs D+Sp500, D+Sp250 vs D+Sp500, D+Sp500 vs SpC group.

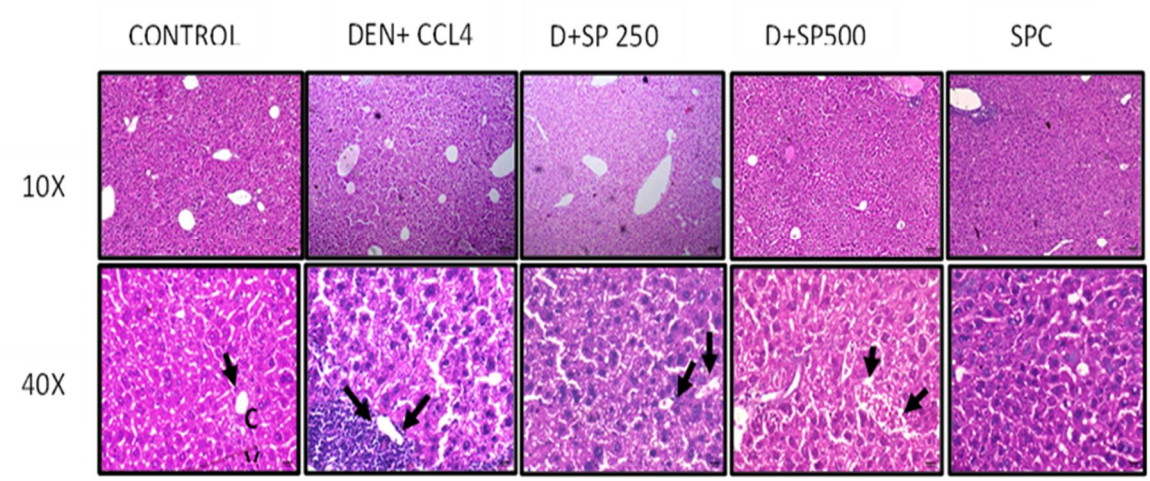

Fig 3. Histopathological examine of liver of different groups of mice (Control, DEN+CCl4, D+Sp250, D+Sp500, SpC group) at 10x and 40x.

CONCLUSION

Our study has shown that administering a higher dose of Spirulina treatment had a more pronounced hepatoprotective impact against hepatocellular carcinoma (HCC) compared to administering a lower dose of Spirulina treatment. The hepatoprotective effect can be linked to the antioxidant characteristics of the Spirulina, as well as its potentiality to reduce oxidative stress and its potentiality to downregulate the expression of genes related to cancer progression and inflammation. Based on the interpretation of this study, it is likely that the application of spirulina as a therapeutic agent might prove to be advantageous in the management of the progression of liver cell carcinoma.

ACKNOWLEDGEMENTS

We gratefully acknowledge and appreciate the UGC non- NET fellowship for financial support and the DST-FIST sponsored Department of Zoology, Dr. Harisingh Gour Vishwavidyalaya, Sagar, M.P. 470003, India for instrumentation.

CONFLICT OF INTEREST STATEMENT.

The authors declare that there is no conflict of interest in the publication of this article.

AUTHORS' CONTRIBUTIONS

All authors contributed equally to this work.

FUNDING

This study was supported by the UGC non- NET fellowship.

CONSENT FOR PUBLICATION

Not applicable.

DATA AVAILABILITY

Data are available upon request.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

All applicable institutional and/or national guidelines for the care and use of animals were followed.

REFERENCES

1. Hamzawy, M. A., El-Denshary, E. S., & Abdel-Wahhab, M. A. (2015). Effects of natural compounds in treatment and prevention of hepatotoxicity and hepatocellular carcinoma. *Hepatoma Research*, *1*, 111-118.
2. Jemal, A., Bray, F., Center, M. M., Ferlay, J., Ward, E., & Forman, D. (2011). Global cancer statistics. *CA: a cancer journal for clinicians*, *61*(2), 69-90.
3. Marquardt, J. U., Galle, P. R., & Teufel, A. (2012). Hepatocellular carcinoma: molecular pathogenesis and novel targets for therapy. *Deutsche Medizinische Wochenschrift (1946)*, *137*(16), 855-860.

4. Poon, D., Anderson, B. O., Chen, L. T., Tanaka, K., Lau, W. Y., Van Cutsem, E., ... & Koo, W. H. (2009). Management of hepatocellular carcinoma in Asia: consensus statement from the Asian Oncology Summit 2009. *The lancet oncology*, *10*(11), 1111-1118.
5. Talari, M., Seydi, E., Salimi, A., Mohsenifar, Z., Kamalinejad, M., & Pourahmad, J. (2014). Dracocephalum: novel anticancer plant acting on liver cancer cell mitochondria. *BioMed research international*, *2014*.
6. Tabone, M., & Pellicano, R. (2006). Prevention of intrahepatic hepatocarcinoma recurrence in patients with viral cirrhosis: two potential options. *Minerva Gastroenterologica e Dietologica*, *52*(1), 47-52.
7. Amin, A., Hamza, A. A., Bajbouj, K., Ashraf, S. S., & Daoud, S. (2011). Saffron: a potential candidate for a novel anticancer drug against hepatocellular carcinoma. *Hepatology*, *54*(3), 857-867.
8. Yamada, K. I., Yamamiya, I., & Utsumi, H. (2006). In vivo detection of free radicals induced by diethylnitrosamine in rat liver tissue. *Free Radical Biology and Medicine*, *40*(11), 2040-2046.
9. Uehara, T., Pogribny, I. P., & Rusyn, I. (2014). The DEN and CCl4-induced mouse model of fibrosis and inflammation-associated hepatocellular carcinoma. *Current protocols in pharmacology*, *66*(1), 14-30.
10. Almatroodi, S. A., Anwar, S., Almatroudi, A., Khan, A. A., Alrumaihi, F., Alsahli, M. A., & Rahmani, A. H. (2020). Hepatoprotective effects of garlic extract against carbon tetrachloride (CCl4)-induced liver injury via modulation of antioxidant, anti-inflammatory activities and hepatocyte architecture. *Applied Sciences*, *10*(18), 6200.
11. Srivastava, A., & Shivanandappa, T. (2010). Hepatoprotective effect of the root extract of Decalepis hamiltonii against carbon tetrachloride-induced oxidative stress in rats. *Food chemistry*, *118*(2), 411-417.
12. Ande, S. R., Nguyen, K. H., Grégoire Nyomba, B. L., & Mishra, S. (2016). Prohibitin-induced, obesity-associated insulin resistance and accompanying low-grade inflammation causes NASH and HCC. *Scientific reports*, *6*(1), 23608.
13. Fu, Y., & Chung, F. L. (2018). Oxidative stress and hepatocarcinogenesis. *Hepatoma research*, *4*.
14. Sasaki, Y. (2006). Does oxidative stress participate in the development of hepatocellular carcinoma? *Journal of gastroenterology*, *41*, 1135-1148.
15. Brown, N. S., & Bicknell, R. (2001). Hypoxia and oxidative stress in breast cancer Oxidative stress-its effects on the growth, metastatic potential and response to therapy of breast cancer. *Breast cancer research*, *3*, 1-5.
16. Polimeni, L., Del Ben, M., Baratta, F., Perri, L., Albanese, F., Pastori, D., ... & Angelico, F. (2015). Oxidative stress: New insights on the association of non-alcoholic fatty liver disease and atherosclerosis. *World journal of hepatology*, *7*(10), 1325.
17. Polimeni, L., Del Ben, M., Baratta, F., Perri, L., Albanese, F., Pastori, D., ... & Angelico, F. (2015). Oxidative stress: New insights on the association of non-alcoholic fatty liver disease and atherosclerosis. *World journal of hepatology*, *7*(10), 1325.
18. Ismail, M. F., Ali, D. A., Fernando, A., Abdraboh, M. E., Gaur, R. L., Ibrahim, W. M., ... & Ouhtit, A. (2009). Chemoprevention of rat liver toxicity and carcinogenesis by Spirulina. *International journal of biological sciences*, *5*(4), 377.
19. Chamorro, G., Salazar, M., Araujo, K. G., dos Santos, C. P., Ceballos, G., & Castillo, L. F. (2002). Update on the pharmacology of Spirulina (Arthrospira), an unconventional food. *Archivos latinoamericanos de nutrición*, *52*(3), 232-240.
20. Grawish, M. E. (2008). Effects of Spirulina platensis extract on Syrian hamster cheek pouch mucosa painted with 7, 12-dimethylbenz [a] anthracene. *Oral Oncology*, *44*(10), 956-962.
21. Abass, S. A., Abdel-Hamid, N. M., Abouzed, T. K., & El-Shishtawy, M. M. (2018). Chemosensitizing effect of Alpinia officinarum rhizome extract in cisplatin-treated rats with hepatocellular carcinoma. *Biomedicine & Pharmacotherapy*, *101*, 710-718.
22. Elaidy, S. M., Moghazy, A., & El-Kherbetawy, M. K. (2017). Evaluation of the therapeutic effects of Polyvinylpyrrolidone-capped silver nanoparticles on the Diethylnitrosamine/carbon tetrachloride-induced hepatocellular carcinoma in rats. *Egyptian Journal of Basic and Clinical Pharmacology*, *7*(2), 9-24.
23. Abdel-Daim, M. M., Abuzead, S. M., & Halawa, S. M. (2013). Protective role of Spirulina platensis against acute deltamethrin-induced toxicity in rats. *Plos one*, *8*(9), e72991.
24. Chatterjee, T. K. (1993). Handbook of laboratory Mice and Rats. *Department of Pharmaceutical Technology, Jadavpur University*, *157*.
25. Reitman, S., & Frankel, S. (1957). A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. *American journal of clinical pathology*, *28*(1), 56-63.
26. Gornall, A. G., Bardawill, C. J., & David, M. M. (1949). Determination of serum proteins by means of the biuret reaction. *J. biol. Chem*, *177*(2), 751-766.
27. Ohkawa, H., Ohishi, N., & Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical biochemistry*, *95*(2), 351-358.
28. Nishikimi, M., Rao, N. A., & Yagi, K. (1972). The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. *Biochemical and biophysical research communications*, *46*(2), 849-854.
29. Aebi, H. (1984). [13] Catalase in vitro. In *Methods in enzymology* (Vol. 105, pp. 121-126). Academic press.
30. Bancroft, J. D., Layton, C., & Suvarna, S. K. (2013). *Bancroft's theory and practice of histological techniques*. Churchill Livingstone Elsevier.
31. Deshwal, N., Sharma, A. K., & Sharma, P. (2011). Review on hepatoprotective plants. *Int J Pharm Sci Rev Res*, *7*, 15-26.
32. Gey, K. F. (1993). Prospects for the prevention of free radical disease, regarding cancer and cardiovascular disease. *British Medical Bulletin*, *49*(3), 679-699.

33. Bulle, F., Mavier, P., Zafrani, E. S., Preaux, A. M., Lescs, M. C., Siegrist, S., ... & Guellaën, G. (1990). Mechanism of γ-glutamyl transpeptidase release in serum during intrahepatic and extrahepatic cholestasis in the rat: A histochemical, biochemical and molecular approach. *Hepatology*, *11*(4), 545-550.
34. Hassan, H. A., El-Gharib, N. E., & Azhari, A. F. (2016). Role of natural antioxidants in the therapeutic management of hepatocellular carcinoma. *Hepatoma Res*, *2*, 216-23.
35. Vásquez-Garzón, V. R., Arellanes-Robledo, J., García-Román, R., Aparicio-Rautista, D. I., & Villa-Treviño, S. (2009). Inhibition of reactive oxygen species and pre-neoplastic lesions by quercetin through an antioxidant defense mechanism. *Free radical research*, *43*(2), 128-137.
36. Whitby, L. G., Percy-Robb, I. W., & Smith, A. F. (1984). Enzymes test in diagnosis. *Lecture notes on Clinical Chemistry*, 138-168.
37. Ramakrishnan, G., Augustine, T. A., Jagan, S., Vinodhkumar, R., & Devaki, T. (2007). Effect of silymarin on N-nitrosodiethylamine induced hepatocarcinogenesis in rats. *Experimental oncology*.
38. A. Almatroodi, S., A. Alsahli, M., Alharbi, H. M., Khan, A. A., & Husain Rahmani, A. (2019). Epigallocatechin-3-gallate (EGCG), an active constituent of green tea: implications in the prevention of liver injury induced by diethylnitrosamine (DEN) in rats. *Applied Sciences*, *9*(22), 4821.
39. Mahmoud, Y. I., Shehata, A. M., Fares, N. H., & Mahmoud, A. A. (2021). Spirulina inhibits hepatocellular carcinoma through activating p53 and apoptosis and suppressing oxidative stress and angiogenesis. *Life Sciences*, *265*, 118827.
40. Khan, Z., Bhadouria, P., & Bisen, P. S. (2005). Nutritional and therapeutic potential of Spirulina. *Current pharmaceutical biotechnology*, *6*(5), 373-379.
41. El-Tantawy, W. H. (2016). Antioxidant effects of Spirulina supplement against lead acetate-induced hepatic injury in rats. *Journal of traditional and complementary medicine*, *6*(4), 327-331.

CITATION OF THIS ARTICLE

Neelam, Priyanka Gupta and Versha Sharma. The Chemoprevention of *Arthrospira platensis* against Diethyl nitrosamine induced Hepatocellular Carcinoma via Amelioration of Oxidative Stress in Mice. Bull. Env.Pharmacol. Life Sci., Vol 13 [4] March 2024: 177-184