Effects of the marine microalga (spirulina) on protein deficiency signs in hepatocellular carcinoma-bearing mice

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INTRODUCTION

Marine microorganisms are sources of a massive amount of bioactive compounds with remarkable pharmaceutical activities. Among these marine microorganisms is spirulina, an edible blue-green microalga that is known to be rich in numerous bioactive compounds especially proteins. Protein deficiency is associated with various disease conditions in human, including cancer. The present study was carried out to evaluate the effect of the marine microalga “spirulina” on protein deficiency signs in hepatocellular carcinoma-bearing mice. Hepatocarcinoma was induced by a single injection of diethylnitrosamine (100mg/kg, intraperitoneally) followed by 22 weekly injections of carbon-tetrachloride (0.5 mg/kg, i.p). Spirulina (250 and 500 mg/kg bw) was given orally, from week 25 to 28, after the establishment of hepatocellular carcinoma. The results showed that HCC-bearing animals several clinical signs of protein deficiency including weakness and skeletal muscle loss, decreased body weight, hair thinning and loss, in addition to abdominal edema. On the other hand, spirulina-treated animals recovered the signs of HCC-associated protein deficiency. In conclusion, spirulina proved to be an adequate protein source to ameliorate the protein deficiency-related alterations in HCC-bearing animals.
because of its nutritional value and relative safety \cite{Smiezeck2017}, and its digestability as it lacks hard cellulose that is present in other plants \cite{Moorhead2011}.

Spirulina contains approximately 70% easily digestible protein where 18 of 22 amino acids and all of the essential amino acid are available, making it a unique vegetarian source of complete protein \cite{Dillon1995}. This nutritious food is an excellent source of B vitamins, and contains vitamin E, a highly bioavailable source of iron, 14 naturally chelated minerals and numerous trace elements \cite{Dillon1995,Somchit2007}. Due to its nutritional value, spirulina has been used in diets of aquatic organisms as a possible replacement of fishmeal, and as a functional feed to improve the growth parameters and general health of fish in the area of aquaculture \cite{Rosas2018}. Spirulina is also used in treating Protein Energy Malnutrition (PEM) and skeletal muscle wasting associated with various disease conditions in human, including cancer \cite{SivaKiran2015}. Treating PEM with good nutrition ameliorates many cancer-associated clinical signs and increases the survival rate of these patients \cite{Nixon1980,SivaKiran2015}. Thus, the current study was designed to detect the potential ameliorative effects of spirulina on the body weight and protein-deficiency signs in hepatocellular carcinoma-bearing mice.

**MATERIALS AND METHODS**

**Drugs and chemicals** Diethylnitrosamine (DEN) was purchased from Sigma-Aldrich (0.95 g/ml; Sigma N0258-1G; St. Louis, MO, USA). Carbon tetrachloride was obtained from Research Lab (Cairo, Egypt). Spirulina, was obtained in commercial form as fine dark blue-green dried powder from Imtenan Healthy Shop (Obour City, Cairo, Egypt). All other chemicals were obtained from El-Nasr pharmaceutical chemicals co. (Cairo, Egypt).

**Experimental animals** Eighty healthy male albino mice \textit{(Mus musculus)}, eight-week-old, weighing 23-28g, were obtained from the Biological Unit of Theodore Bilharz Institute (Giza, Egypt). Mice were acclimatized to laboratory conditions for a week before starting the experiments. A temperature of 25°C and 12 h light/dark cycle were maintained. The animals had free access to water and standard chow pellets. The experimental protocol was approved by the Research Committee of Zoology Department, Faculty of Science, Ain Shams University.

**Induction of hepatocellular carcinoma** HCC was induced as described by \cite{Uehara2013}. Briefly, diethylnitrosamine (DEN; 100 mg/kg in 0.9% saline) was injected intraperitoneally. Two weeks later, the animals received repeated biweekly intraperitoneal injections of carbon tertrachloride (0.5 ml/kg dissolved in corn oil) up to 22 weeks.
**Experimental design** The animals were randomly assigned to five groups, as follows: Group 1 (n=10): received the vehicle and served as negative control group; Group 2 (n=10): received Spirulina (500 mg/kg bw) for 4 weeks by a gastric gavage, and served as Spirulina-control group; Group 3 (n=20): HCC-control group. Group 4 and 5 (n=20): were treated with Spirulina (at dose of 250 and 500 mg/kg b.w, respectively) for 4 weeks, starting from week 25 until week 28 of HCC induction. At the end of the experiment, all mice were examined for clinical signs of protein deficiency such as weakness and muscle wasting, and body weight decrease, fur changes, and abdominal edema.

**Statistical analysis** Numerical data were expressed as mean values and standard error of means. GraphPad Prism (version 5.0, GraphPad software, San Diego, CA, USA) was used to conduct all statistical analysis. Data were analysed statistically using One-way ANOVA followed by post hoc multiple comparisons (Tukey’s test) for comparative analysis between the groups. \( P<0.05 \) was regarded as statistically significant.

**RESULTS**

At the end of the experiment, non-treated (negative control) mice appeared healthy and active, with white and soft fur (Figure 1a) and significant increase in body weight (Table1). When treating normal mice with spirulina, the animals showed no significant changes in their appearance or their behavior; however, they had slightly insignificant decreased body weight compared with that of the blank control group (Table 1). On the other hand, HCC-bearing animals became hypoactive and lethargic, and suffering from abdominal edema (Figure 1c). Their fur was light, matted, and slightly yellowish (Figure 1b). The final weights of HCC animals were significantly lower compared with the average weight gain of the negative control group as shown in Table 1. Treating HCC animals with spirulina resulted in recovery from the clinical symptoms of HCC. Although spirulina-treated HCC animals showed significant decreased body weight compared with HCC control ones; however, the mice were relatively active, with soft white fur and no abdominal edema (Figs. 1d,e; table 1).

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**Figure 1: Effect of spirulina on hepatocellular carcinoma-bearing mice**
Table 1: Effect of spirulina on body weight in hepatocellular carcinoma-bearing mice

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal</th>
<th>Spirulina Control</th>
<th>HCC mice</th>
<th>Spirulina Control</th>
<th>Spirulina 250mg/kg</th>
<th>Spirulina 500mg/kg</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Final</td>
<td>Initial</td>
<td>Final</td>
<td>Initial</td>
<td>Final</td>
</tr>
<tr>
<td>Average body weights</td>
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<td>43.69</td>
<td>22.23</td>
<td>38.87</td>
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<tr>
<td>% b. wt change</td>
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<td>76.41</td>
<td>72.90</td>
<td>54.14</td>
<td>49.71</td>
<td></td>
</tr>
</tbody>
</table>

Values are expressed as means ± SEM.

a indicates the significant difference of HCC control group vs. the corresponding normal group at P<0.05.

b indicates the significant difference of spirulina-treated groups vs. HCC control group at P<0.05.

DISCUSSION

The present study was carried out to evaluate the effect of spirulina on clinical signs of protein deficiency in murine hepatocellular carcinoma. At the end of the current study (week 28 after HCC induction), several signs of protein deficiency appeared in surviving HCC-bearing mice. The first sign of protein deficiency in HCC animals was the weakness and loss of skeletal muscles- the body’s largest reservoir of protein. Luo et al. (2013) also observed that HCC mice became lethargic and lean, 20 weeks after the initiation of HCC induction. The same group of animals also showed significantly decreased body weight compared with that of the negative control group, as reported earlier by Abdo et al. (2015), Kumar et al. (2016), Wang et al. (2016), Fathy et al. (2017), and Ding et al. (2019). This decrease in body weight gain was associated with abdominal edema, another sign of protein deficiency. One of protein’s main functions is to maintain oncotic pressure that draws fluid into the blood circulation and prevents excessive amounts of fluid from accumulating in tissues. Severe protein deficiency leads to lower oncotic pressure, resulting in fluid accumulation in the tissues and abdominal cavity (Singh and Seth, 2017).

Furthermore, HCC-bearing animals in the current study suffered from many fur changes including hair thining, dryness and loss. Luo et al. (2013) and Wang et al. (2016) also mentioned that the fur of HCC mice was matted and lusterless, and turned from white, soft, and fine to yellow, hard, and crude. Since protein is the main structural component of hair, so, protein malnutrition can result in hair thinning and loss (McLaren, 1987; Guo and Katta, 2017).

Spirulina-treated animals showed signs of recovery from the weakness, fur lightness and loss, and abdominal edema that result from severe protein deficiency. These observations could be explained in the light of the high protein content of spirulina (Dillon et al., 1995). In conclusion, spirulina proved to be an adequate protein source to ameliorate the protein deficiency-related alterations in HCC-bearing animals.
Competing interests

We declare that we have no conflict of interest. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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REFERENCES


ARABIC SUMMARY

تأثير الطحالب البحرية الدقيقة (Spirulina) على علامات نقص البروتينات في الفئران المصابة بسرطان الكبد

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تعتبر النشطة بيولوجيا وخاصة البروتينات. يرتبط نقص البروتين بالعديد من الحالات المرضية في الإنسان، بما فيها ذلك السرطان. أجربت هذه الدراسة لتحقيق تأثير الطحالب البحرية الصغيرة "Spirulina" على علامات نقص البروتينات في الفئران المصابة بسرطان الكبد. تم إجراء سرطان الكبد عن طريق حقن ثانائي إيثيل نيتروزامين (100 مجم/كجم) مرة واحدة تليها 22 حقنة أسبوعية من رابع كلوريد الكربون (0.5 مجم/كجم). أعطت "Spirulina" (250 و500 ملجم/كجم من وزن الجسم) عن طريق الفم، من الأسبوع 25 إلى 28، بعد إحداث سرطان الخلايا الكبدية. أوضحت النتائج أن الحيوانات المصابة بسرطان الكبد أظهرت العديد من العلامات السريرية لنقص البروتين بما في ذلك ضعف العضلات، وانخفاض وزن الجسم، وفقدان الشعر، بالإضافة إلى الاستشفاء. من ناحية أخرى، فإن الحيوانات المعالجة بالسبيروكلين تمتاعت من علامات نقص البروتين المرتبطة بسرطان الكبد، وفي الختام، أثبتت السبيروكلين أنها مصدر بروتين مناسب لتحسين التغييرات المتعلقة بنقص البروتينات في الحيوانات المصابة بسرطان الكبد.