World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i7.1085

World J Gastroenterol 2013 February 21; 19(7): 1085-1090
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

BRIEF ARTICLE

Efficacy and safety of Chlorella supplementation in adults with chronic hepatitis C virus infection

Jose Azocar, Arley Diaz

Jose Azocar, Arley Diaz, Northgate Medical Center, Springfield, MA 01103, United States
Author contributions: Azocar J designed and performed the research, collected and analyzed the data and wrote the paper; Diaz A analyzed data and revised the paper.
Supported by Northgate Medical Center, Research Program NMSC01
Correspondence to: Jose Azocar, MD, DSc, Northgate Medical Center, 1985 Main Street, Springfield, MA 01103, United States. jazocar@rcn.com
Telephone: +1-413-7339955 Fax: +1-413-7889840
Received: September 14, 2012 Revised: December 27, 2012
Accepted: January 17, 2013
Published online: February 21, 2013

Abstract

AIM: To evaluate the safety and efficacy of Chlorella in 18 patients chronically infected with hepatitis C virus (HCV) genotype 1.

METHODS: Eighteen adults with chronic infection by HCV genotype 1 received daily oral supplementation of Chlorella for 12 wk. Changes in the RNA levels of HCV, as well as those of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels were evaluated following this treatment period. Paired *t* tests were conducted to compare the means of the different variables at the beginning and end of the study. Side effects and quality of life aspects were also compared between weeks 0 and 12 of the study period.

RESULTS: A majority 84.61% of the patients had a significant decrease in their ALT levels from week 0 to week 12. Evaluation of side effects showed that Chlorella was well tolerated. Quality of life assessment showed that 76.9 of the participants reported an improvement in their energy levels and 46.1% reported an improvement in their perception of general health. Although 69.23% also showed a decrease in their AST levels, this was not statistically significant. Most patients that exhibited an improvement in their ALT and AST levels also showed a tendency toward a decreased HCV viral load. The HCV RNA levels showed a decrease in 69.23% of the patients, which along with changes in AST/ALT ratios from week 0 to week 12, these results were not statistically significant.

CONCLUSION: Chlorella supplementation was well tolerated in patients with chronic HCV and associated with a significant decrease in ALT liver enzyme levels.

Key words: Chlorella; Hepatitis C virus; Interferon; Aspartate and alanine aminotransferase; Ratio

Azocar J, Diaz A. Efficacy and safety of Chlorella supplementation in adults with chronic hepatitis C virus infection. *World J Gastroenterol* 2013; 19(7): 1085-1090 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i7/1085.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i7.1085

INTRODUCTION

Complementary and alternative medicines to treat chronic liver diseases, including chronic hepatitis C virus (HCV) infection, are becoming increasingly popular in North America[1-5]. Infection with HCV is global in nature, infecting approximately 160 million persons worldwide or roughly 2% of the world population, with some countries documenting a rate of 15% or more[6,7]. After an initial HCV infection, close to 70% of cases develop chronic infection that may progress to liver cirrhosis and hepatocellular carcinoma if left untreated[8]. The successful treatment of chronic HCV infection is determined by a reduced HCV-RNA viral load and improved liver function and histology. The current Food and Drug

Administration approved treatment for HCV is up to 42 wk of interferon and antiviral medications. However, there are significant costs associated with these medications and side effects which limit their use thus stressing the need for novel treatment options[9,10]. In addition, subjects that fail to respond to the initial treatment are less likely to respond to retreatment[11]. Many herbal and other natural compounds have now been used for the treatment of liver diseases, including HCV infection. Silybum marianum and Lactoferrin have been associated with a decrease in serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels[12,13]. More recently, green tea catechins have been shown to inhibit HCV attachment and transmission in human liver cells *in vitro*[14]. However, the potential benefits of herbal and other natural molecules in inhibiting the progression of HCV infection are only beginning to be understood, and more controlled studies are needed in this area.

Chlorella, a fresh water unicellular alga rich in macro- and micronutrients, has been used as a food source and nutritional supplement for centuries[15]. In animals, Chlorella has been reported to improve host resistance to viral infection and tumors[16-18]. In humans, Chlorella supplementation has been shown to enhance the antibody titer after influenza immunization[19], and to improve the outcome in several chronic diseases[20,21]. Chlorella supplementation has been also associated with an improvement in liver function in animal models[22,23]. Due to the documented benefits of Chlorella treatments in liver diseases in animals and in chronic diseases in humans, we studied the effects of Chlorella in 18 patients with chronic HCV genotype 1 infection. The plasma HCV RNA levels, hematological and chemistry results, including liver enzyme levels, and the quality of life and psychological well-being were assessed in this cohort following dietary supplementations with Chlorella-derived products.

MATERIALS AND METHODS

Population

The current study trial took place at a primary care clinic in western Massachusetts. Approval was obtained from the New England Institutional Review Board (NEIRB, Wellesley, MA, United States). The study cohort comprised 18 patients with chronic HCV infection who were either unwilling or unable to receive an interferon plus antiviral therapy. The inclusion criteria were an age of 18-65 years, evidence of chronic HCV infection by reverse transcription polymerase chain reaction (PCR), and a confirmation that the infection was due to HCV genotype 1. The exclusion criteria were any acute or chronic liver disease other than chronic HCV infection, any evidence of advanced liver disease such as a history or presence of ascites, a history of bleeding esophageal varices or encephalopathy, any known existing medical condition that could interfere with participation in the current study, co-infection with hepatitis B virus and/or human immunodeficiency virus, or a history of active alcohol or drug abuse three months prior to the beginning of the trial. Some of the patients had already been treated with interferon plus ribavirin (three years or more previously) before their participation in this study and had failed to respond.

Study design

This study examined the effects of Chlorella upon the HCV viral load, and on hematological and chemical test results, including AST and ALT liver enzyme levels, in infected patients during a 12 wk treatment period. To control for factors others than Chlorella that could have affected the results of our study, only patients with a HCV genotype 1 infection were selected for this trial. The AST/ALT ratio at week 0 and after the 12 wk of Chlorella supplementation was compared among the 13 subjects in the cohort.

We also compared the safety and efficacy of orally administered Chlorella by assessing the presence of possible side effects and their impact on quality of life. The side effects assessed included constipation, diarrhea, depression, irritability, headache/body aches, and any other significant symptoms that arose during the study period. Similarly, quality of life was assessed by evaluating changes in energy levels, general health perceptions, quality of sleep and changes in appetite. Patients were interviewed about possible side effects and impact of quality of life at baseline, and at weeks 1, 2, 4, 8 and 12. The answers were coded on a 5 level scale from 1 (much worse) to 5 (much better). Scores were then compared between baseline and weeks 1, 2, 4, 8 and 12. To assess compliance, patients were instructed to return all used and unused products at each visit.

"Sun Chlorella A™" consisted of a dry pulverized Chlorella pyrenoidosa powder plus a water soluble extract ("Wakasa Gold™"; Sun Chlorella Corp, Kyoto, Japan), which contained 82 mg/mL of Chlorella. It was administered orally to each patient as follows: three 500 mg tablets were administered twice daily on days 1-7 and then three times each day thereafter. Wakasa Gold ™ was administered at a dose of 30 mL twice a day starting on day 1. Both pulverized tablets and water soluble extracts were used in the treatment given in order to administer the greatest amount of Chlorella possible. Patients were evaluated as described above during the duration of the trial. Routine hematology, chemical tests and HCV RNA levels were done at baseline and at week 12 using a PCR-based assay conducted at a local laboratory (Life Lab; Mercy Hospital, Springfield, MA, United States).

Changes in AST and ALT liver enzyme levels and the HCV viral load in our "Chlorella treat" cohort were measured after the 12 wk study period and were compared with the same laboratory test results in a control group of 26 subjects who were also chronically infected with HCV genotype 1, but did not receive Chlorella. Subjects

Table 1 Levels of aspartate aminotransferase, alanine aminotransferase, aspartate/alanine aminotransferase ratio and hepatitis C virus viral load at weeks 0 and 12

Patient No.	AST (IU/mL)		ALT (IU/mL)		AST/ALT ratio		HCV viral load (IU/mL)		Previous
	Week 0	Week 12	Week 0	Week 12	Week 0	Week 12	Week 0	Week 12	INF Rx
1	80	63	82	68	0.975	0.926	11 420	1183	No
2	53	44	103	72	0.514	0.611	140 642	32 334	No
3	87	40	71	39	1.225	1.025	91 808	24 141	No
4	35	56	48	92	0.729	0.608	223 075	89 331	No
5	19	25	22	21	0.863	1.19	221 116	281 886	No
6	39	33	83	72	0.469	0.454	1 917 040	560 082	No
7	51	41	81	64	0.629	0.645	269 079	217 334	Yes
8	138	74	157	105	0.878	0.704	382 773	1 302 860	Yes
9	48	51	37	47	1.297	1.085	136 292	236 748	Yes
10	75	85	102	95	0.735	0.894	716 152	503 764	Yes
11	27	24	39	29	0.692	0.827	7 672 080	7 692 310	Yes
12	103	77	157	119	0.656	0.647	847 412	542 398	Yes
13	127	93	159	106	0.798	0.877	869 846	568 398	Yes

AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; HCV: Hepatitis C virus; INF: Interferon.

in the control group were randomly selected, attended the same clinic and had a similar demographic profile as the Chlorella-treated group. Changes in the hematological, liver enzyme values and HCV-RNA viral load in this control group were assessed within an 11-21 wk period as part of a standard clinical evaluation. No subjects in either the study or control groups received treatment with interferon or other anti-viral drugs during the study period. This study was conducted in accordance with the Declaration of Helsinki, Good Clinical Practices and the study protocol was reviewed and approved by the NEIRB.

Statistical analysis

Data gathered during the study was used to examine statistically significant changes in the HCV RNA levels, and in the hematological and liver enzyme (AST, ALT) levels. Side effects and quality of life aspects were also compared between weeks 0 and 12 of the study period. Paired *t* tests were conducted to compare the means of the different variables at the beginning and end of the study. *P* values < 0.05 were considered statistically significant. All data was analyzed using Statistical Package for Social Sciences version 17.0.

RESULTS

Thirteen out of 18 enrolled patients completed our 12 wk study. Of the five patients who did not complete the trial, one individual discontinued treatment due to constipation on the first two days of treatment, which resolved upon treatment withdrawal. Four subjects were excluded due to poor compliance which was unrelated to side effects or changes in their general health status.

HCVRNA titer and liver enzyme analysis

Changes in the HCV RNA levels and in the AST and ALT liver enzyme levels among our study subjects are shown in Table 1. A majority of patients experienced an improvement in their liver enzyme profiles; 84.61% (11/13) had a decrease in their ALT levels from week 0 (mean ± SD, 89.30% ± 49.36%) to week 12 (mean ± SD, 71.46% ± 31.12%). Moreover, this decrease was statistically significant ($P < 0.05$). Although 69.23% of the patients (9/13) also showed a decrease in their AST levels from week 0 (mean ± SD, 67.84% ± 37.73%) to week 12 (mean ± SD, 54.30% ± 22.63%), this was not statistically significant ($P = 0.06$). The results further showed that 69.23% (9/13) of the patients had a decrease in their HCV RNA levels, although the results of the paired *t* test also showed this was not statistically significant ($P = 0.42$). Most patients that exhibited an improvement in their AST and ALT levels also showed a tendency toward a decreased HCV viral load. An exception was patient No. 8 whose viral load increased significantly (Table 1). The changes in the AST/ALT ratio between weeks 0 and 12 among the subjects in the Chlorella cohort shown in Table 1 were not statistically significant. The values obtained from additional laboratory tests, including routine hematology (complete blood count and differential) and chemical tests, showed no statistically significant changes during the study period (data not shown). No statistically significant differences in the changes of the AST and ALT liver enzymes, AST/ALT ratio and HCV viral load was observed within a 11-21 wk period, among the control group who received not Chlorella supplementation.

Side effect profile and quality of life assessment

The main side effects associated with the Chlorella treatments in our trial included constipation and diarrhea. Four of the 13 patients (30.7%) with a previous history of constipation reported their symptoms as worse or somewhat worse during the first two weeks of treatment. However, in all of these cases, the constipation symptoms were mild to moderate and resolved within the first two weeks. These patients continued in the trial for the scheduled 12 wk. Similarly, two of the 13 patients (15.3%) who completed the study complained of mild

diarrhea at week 1, but reported that these symptoms were much improved after week 2. None of the patients reported symptoms of abdominal pain, fever, depression, headache, body ache or other complaints during the 12 wk study.

Quality of life

Four variables were tested to assess changes in quality of life; 76.9 % of the patients (10/13) reported an improvement in their energy levels during the study period whereas 23.1% (3/13) reported no change in energy levels. In addition, 46.1% (6/13) of the patients reported an improvement in their perception of general health while 53.9% (7/13) reported no change in this regard. None of the patients reported issues with sleep quality or appetite during the 12 wk study period.

DISCUSSION

In our present study, most of the subject patients with chronic HCV showed a good tolerance to Chlorella oral supplementation. This was anticipated, based on previous studies which had also shown good tolerance of similar doses of Chlorella administered in pregnant, lactating[24], and elderly subjects[19], as well as in patients with diverse chronic diseases[20,21]. In addition, a significant percentage of our subjects reported health improvements in their quality of life questionnaire: 76.9% had an increase in energy levels and 46.1 % described an improvement in their general health perception.

The most significant finding from our current study was the statistically significant decrease in ALT levels, a marker of liver inflammation, among our patient cohort after 12 wk of Chlorella treatment. The cause-effect relationship between the observed significant decrease in the ALT levels and treatment with Chlorella is further suggested by the lack of significant ALT changes within the 11-21 wk period in a control group of HCV genotype 1 infected patients who had not received Chlorella. We further found that 69.23% of our patients showed a tendency toward a decrease in both their AST and HCV RNA levels after the 12 wk Chlorella treatment period, although this was not statistically significant. The AST/ALT ratio has been widely used as an indicator of liver disease; a ratio higher of 1 is used as indicator of liver fibrosis and cirrhosis[25,26]. In our cohort only four of thirteen subjects had an AST/ALT higher than 1 and there was no significant change after the Chlorella supplementation period (Table 1).

Animal studies have shown that enhanced immunocompetence provided by oral administration of Chlorella extract is mediated by augmentation of cell mediated immunity in normal and immunocompromised hosts[16-18]. More recently Kwak *et al*[27] demonstrated enhancement of natural killer cell activity and increase interferon and other cytokines production in humans after an 8 wk period of Chlorella supplementation at doses similar to the ones used in this study. We propose that the improvements in liver function tests in our population with chronic HCV infection is most likely due to the beneficial immunostimulatory effect of Chlorella supplementation. This is consistent with the finding that in our cohort most of the subjects that had been previously treated with interferon and failed to respond, were less likely to show a decrease in ALT and HCV-RNA values during Chlorella supplementation (Previous INF Rx, Table 1). The recovery from liver inflammation in all viral and non-viral cases of hepatitis is associated with a reduction in the ALT levels[28-30]. In Chronic HCV infection, early normalization of the ALT levels is predictive of the response to interferon[9,10,30]. However, whether the significant decrease in ALT values observed in our patients after 12 wk of Chlorella administration is associated with an improvement in liver histology remains to be determined.

The strength of our hypothesis; that the benefits of Chlorella supplementation are related to the well known immunoenhancement effects of Chlorella is limited by the lower number of subjects in our cohort and the limited time of supplementation (12 wk). Nevertheless, we conclude from our present findings that the benefits of Chlorella administration in the treatment of chronic HCV infection before and/or during the administration of interferon plus antiviral drugs, as well as the effects of Chlorella upon chronic infection by other HCV genotypes, warrant further study.

ACKNOWLEDGMENTS

The authors acknowledge the excellent editorial support of Camille Alvarado-Pinguelo, MPH. Sun Chlorella A™ and Wakasa Gold™ were generously provided by Sun Chlorella Corporation (Kyoto, Japan).

COMMENTS

Background

Chronic hepatitis C viral (HCV) infection are becoming increasingly popular in North America and most cases develop chronic infection that may progress to liver cirrhosis and hepatocellular carcinoma if left untreated. Chlorella supplementation has been shown to enhance the antibody titer after influenza immunization, and to improve the outcome in several chronic diseases.

Research frontiers

Animal studies have shown that enhanced immunocompetence provided by oral administration of Chlorella extract is mediated by augmentation of cell mediated immunity in normal and immunocompromised host. More recently Kwak *et al* demonstrated enhancement of natural killer cell activity and increase interferon and other cytokines production in humans after an 8 wk period of Chlorella supplementation at doses similar to the ones used in this study.

Innovations and breakthroughs

The study suggestes that the benefits of Chlorella administration in the treatment of chronic HCV infection before and/or during the administration of interferon plus antiviral drugs, as well as the effects of Chlorella upon chronic infection by other HCV genotypes, warrant further study.

Terminology

Chlorella, a fresh water unicellular alga rich in macro- and micronutrients, has been used as a food source and nutritional supplement for centurie. In animals,

Chlorella has been reported to improve host resistance to viral infection and tumors.

Peer review

The results are interesting and suggest that most of the subject patients with chronic HCV showed a good tolerance to Chlorella oral supplementation.

REFERENCES

1 **Modi AA**, Wright EC, Seeff LB. Complementary and alternative medicine (CAM) for the treatment of chronic hepatitis B and C: a review. *Antivir Ther* 2007; **12**: 285-295 [PMID: 17591018]

2 **White CP**, Hirsch G, Patel S, Adams F, Peltekian KM. Complementary and alternative medicine use by patients chronically infected with hepatitis C virus. *Can J Gastroenterol* 2007; **21**: 589-595 [PMID: 17853954]

3 **Seeff LB**, Curto TM, Szabo G, Everson GT, Bonkovsky HL, Dienstag JL, Shiffman ML, Lindsay KL, Lok AS, Di Bisceglie AM, Lee WM, Ghany MG. Herbal product use by persons enrolled in the hepatitis C Antiviral Long-Term Treatment Against Cirrhosis (HALT-C) Trial. *Hepatology* 2008; **47**: 605-612 [PMID: 18157835 DOI: 10.1002/hep.22044]

4 **Mollison L**, Totten L, Flexman J, Beaman M, Batey R. Randomized double blind placebo-controlled trial of a Chinese herbal therapy (CH100) in chronic hepatitis C. *J Gastroenterol Hepatol* 2006; **21**: 1184-1188 [PMID: 16824073 DOI: 10.1111/j.1440-1746.2006.04320.x]

5 **Batey RG**, Bensoussan A, Fan YY, Bollipo S, Hossain MA. Preliminary report of a randomized, double-blind placebo-controlled trial of a Chinese herbal medicine preparation CH-100 in the treatment of chronic hepatitis C. *J Gastroenterol Hepatol* 1998; **13**: 244-247 [PMID: 9570235 DOI: 10.1111/j.1440-1746.1998.01550.x]

6 **Shepard CW**, Finelli L, Alter MJ. Global epidemiology of hepatitis C virus infection. *Lancet Infect Dis* 2005; **5**: 558-567 [PMID: 16122679 DOI: 10.1016/S1473-3099(05)70216-4]

7 **Williams I**. Epidemiology of hepatitis C in the United States. *Am J Med* 1999; **107**: 2S-9S [PMID: 10653448 DOI: 10.1016/S0002-9343(99)00373-3]

8 **Seeff LB**, Hollinger FB, Alter HJ, Wright EC, Cain CM, Buskell ZJ, Ishak KG, Iber FL, Toro D, Samanta A, Koretz RL, Perrillo RP, Goodman ZD, Knodell RG, Gitnick G, Morgan TR, Schiff ER, Lasky S, Stevens C, Vlahcevic RZ, Weinshel E, Tanwandee T, Lin HJ, Barbosa L. Long-term mortality and morbidity of transfusion-associated non-A, non-B, and type C hepatitis: A National Heart, Lung, and Blood Institute collaborative study. *Hepatology* 2001; **33**: 455-463 [PMID: 11172349 DOI: 10.1053/jhep.2001.21905]

9 **Poynard T**, Marcellin P, Lee SS, Niederau C, Minuk GS, Ideo G, Bain V, Heathcote J, Zeuzem S, Trepo C, Albrecht J. Randomised trial of interferon alpha2b plus ribavirin for 48 weeks or for 24 weeks versus interferon alpha2b plus placebo for 48 weeks for treatment of chronic infection with hepatitis C virus. International Hepatitis Interventional Therapy Group (IHIT) *Lancet* 1998; **352**: 1426-1432 [PMID: 9807989]

10 **Poordad F**, McCone J, Bacon BR, Bruno S, Manns MP, Sulkowski MS, Jacobson IM, Reddy KR, Goodman ZD, Boparai N, DiNubile MJ, Sniukiene V, Brass CA, Albrecht JK, Bronowicki JP. Boceprevir for untreated chronic HCV genotype 1 infection. *N Engl J Med* 2011; **364**: 1195-1206 [PMID: 21449783 DOI: 10.1056/NEJMoa1010494]

11 **Pomilio AB**, Buschi CA, Tomes CN, Viale AA. Antimicrobial constituents of Gomphrena martiana and Gomphrena boliviana. *J Ethnopharmacol* 1992; **36**: 155-161 [PMID: 1608273 DOI: 10.1186/1471-2334-10-212]

12 **Kalantari H**, Shahshahan Z, Hejazi SM, Ghafghazi T, Sebghatolahi V. Effects of silybum marianum on patients with chronic hepatitis C. *J Res Med Sci* 2011; **16**: 287-290 [PMID: 22091246]

13 **Tanaka K**, Ikeda M, Nozaki A, Kato N, Tsuda H, Saito S, Sekihara H. Lactoferrin inhibits hepatitis C virus viremia in patients with chronic hepatitis C: a pilot study. *Jpn J Cancer Res* 1999; **90**: 367-371 [PMID: 10363572 DOI: 10.1111/j.1349-7006.1999.tb00756.x]

14 **Calland N**, Albecka A, Belouzard S, Wychowski C, Duverlie G, Descamps V, Hober D, Dubuisson J, Rouillé Y, Séron K. (-)-Epigallocatechin-3-gallate is a new inhibitor of hepatitis C virus entry. *Hepatology* 2012; **55**: 720-729 [PMID: 22105803 DOI: 10.1002/hep.24803]

15 **Kay RA**. Microalgae as food and supplement. *Crit Rev Food Sci Nutr* 1991; **30**: 555-573 [PMID: 1741951 DOI: 10.1080/10408399109527556]

16 **Miyazawa Y**, Murayama T, Ooya N, Wang LF, Tung YC, Yamaguchi N. Immunomodulation by a unicellular green algae (Chlorella pyrenoidosa) in tumor-bearing mice. *J Ethnopharmacol* 1988; **24**: 135-146 [PMID: 3253484 DOI: 10.1016/0378-8741(88)90145-6]

17 **Tanaka K**, Konishi F, Himeno K, Taniguchi K, Nomoto K. Augmentation of antitumor resistance by a strain of unicellular green algae, Chlorella vulgaris. *Cancer Immunol Immunother* 1984; **17**: 90-94 [PMID: 6565519 DOI: 10.1007/BF00200042]

18 **Hasegawa T**, Kimura Y, Hiromatsu K, Kobayashi N, Yamada A, Makino M, Okuda M, Sano T, Nomoto K, Yoshikai Y. Effect of hot water extract of Chlorella vulgaris on cytokine expression patterns in mice with murine acquired immunodeficiency syndrome after infection with Listeria monocytogenes. *Immunopharmacology* 1997; **35**: 273-282 [PMID: 9043941 DOI: 10.1016/S0162-3109(96)00150-6]

19 **Halperin SA**, Smith B, Nolan C, Shay J, Kralovec J. Safety and immunoenhancing effect of a Chlorella-derived dietary supplement in healthy adults undergoing influenza vaccination: randomized, double-blind, placebo-controlled trial. *CMAJ* 2003; **169**: 111-117 [PMID: 12874157]

20 **Merchant RE**, Andre CA. A review of recent clinical trials of the nutritional supplement Chlorella pyrenoidosa in the treatment of fibromyalgia, hypertension, and ulcerative colitis. *Altern Ther Health Med* 2001; **7**: 79-91 [PMID: 11347287]

21 **Mizoguchi T**, Takehara I, Masuzawa T, Saito T, Naoki Y. Nutrigenomic studies of effects of Chlorella on subjects with high-risk factors for lifestyle-related disease. *J Med Food* 2008; **11**: 395-404 [PMID: 18800884]

22 **Takekoshi H**, Mizoguchi T, Komasa Y, Chubachi H, Inoue Y, Imanishi H, Nakano M. Suppression of glutathione S-transferase placental form-positive foci development in rat hepatocarcinogenesis by Chlorella pyrenoidosa. *Oncol Rep* 2005; **14**: 409-414 [PMID: 16012723]

23 **Takekoshi H**, Suzuki G, Chubachi H, Nakano M. Effect of Chlorella pyrenoidosa on fecal excretion and liver accumulation of polychlorinated dibenzo-p-dioxin in mice. *Chemosphere* 2005; **59**: 297-304 [PMID: 15722102 DOI: 10.1016/j.chemosphere.2004.11.026]

24 **Nakano S**, Takekoshi H, Nakano M. Chlorella pyrenoidosa supplementation reduces the risk of anemia, proteinuria and edema in pregnant women. *Plant Foods Hum Nutr* 2010; **65**: 25-30 [PMID: 20013055 DOI: 10.1007/s11130-009-0145-9]

25 **Lackner C**, Struber G, Liegl B, Leibl S, Ofner P, Bankuti C, Bauer B, Stauber RE. Comparison and validation of simple noninvasive tests for prediction of fibrosis in chronic hepatitis C. *Hepatology* 2005; **41**: 1376-1382 [PMID: 15915455]

26 **Fouad SA**, Esmat S, Omran D, Rashid L, Kobaisi MH. Noninvasive assessment of hepatic fibrosis in Egyptian patients with chronic hepatitis C virus infection. *World J Gastroenterol* 2012; **18**: 2988-2994 [PMID: 22736923]

27 **Kwak JH**, Baek SH, Woo Y, Han JK, Kim BG, Kim OY, Lee JH. Beneficial immunostimulatory effect of short-term Chlorella supplementation: enhancement of Natural Killer cell activity and early inflammatory response (Randomized, double-blinded, placebo-controlled trial). *Nutr J* 2012; **11**: 53 [PMID: 22849818 DOI: 10.1186/1475-2891-11-53]

28 **Wedemeyer H**, Hofmann WP, Lueth S, Malinski P, Thimme R, Tacke F, Wiegand J. [ALT screening for chronic liver diseases: scrutinizing the evidence]. *Z Gastroenterol* 2010; **48**: 46-55 [PMID: 20072996]

29 **Lee MH**, Yang HI, Lu SN, Jen CL, Yeh SH, Liu CJ, Chen PJ, You SL, Wang LY, Chen WJ, Chen CJ. Hepatitis C virus seromarkers and subsequent risk of hepatocellular carcinoma: long-term predictors from a community-based cohort study. *J Clin Oncol* 2010; **28**: 4587-4593 [PMID: 20855826]

30 **Seeff LB**. Natural history of chronic hepatitis C. *Hepatology* 2002; **36**: S35-S46 [PMID: 12407575 DOI: 10.1053/jhep.2002.36806]

P- Reviewers Zhang XW, Chetty R
S- Editor Zhai HH **L- Editor** A **E- Editor** Xiong L