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Research Article

Cultivation of spirulina (*Arthrospira platensis*) and its use as a food supplement to increase blood hemoglobin level

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Abstract

In our present study to utilize one of the oldest organisms Spirulina (*Arthrospira platensis*) that gave us life, have been cultivated, analyzed and used it as proteinic food supplement in various forms. For cultivating Spirulina, open tank method (Watanabe and Hall) was adopted. Mother culture was obtained from "Lakshmi Spirulina farm and production. Thirubuvani-Puducherry". By practicing and maintaining various mechanical and physical-chemical parameters. Spirulina was obtained in powder form. Microbial analysis of Spirulina was done by performing various tests such as microscopic examination, Gram's staining, motility test and by culturing in various media to check its quality for safe human consumption. It was found that Spirulina contains low molecular weight plasmid DNA and so it can be easily digested by human being. After performing CBC variable analysis among selected subjects, (who have consumed 2-3mg of Spirulina per day for a period of 12 weeks) a difference in the values of each variable between baseline, week 6 and 12 were studied. We observed a significant increase in MCH during supplementation. Over ¾th of the subjects had increased values of HCT, MCV and MCH after 6 weeks. At 12 weeks an increase in WBCs and hemoglobin level was observed in 80% of the subjects. We encourage the design of larger clinical studies with solid endpoints and sufficiently long follow-up with appropriate randomization.

Keywords: Hemoglobin, Hematonic, Spirulina, Cultivation, CBC.

Introduction

Millions of health conscious people around the world called a food of the future only 20 years ago, "Spirulina blue green algae is shaping food pattern as we approach the next century". People are becoming aware that diseases like cancer are directly related to environmental factors. A growing portion of earth's population is seeking super health products against pollution, boost their immune system, resist disease and retard the aging process.

Probiotics, nutraceuticals, phytochemical, designer food – which do we start? With the originated food designed by nature – Spirulina!. To raise our energetic frequency, bring this light food into our life.

Each day new research brings to light the wonders (hidden) in microscopic algae. In 1989, the National Cancer Institute announced sulfolipids extracted from blue green algae were 'remarkably active'

against the AIDS virus in test tube experiments. Sulfolipids can prevent viruses from either attaching to or penetrating into cells, thus preventing viral infection.

Spirulina “The super food “ has the most remarkable concentration of functional nutrients ever known in any food, plant, green or herb.

Cell division

Spirulina multiplies due to presence of hormogonia which helps organisms to multiply by dividing the filaming and arranged one above the other called “trichome”.

It contains essential amino acids [Isoleucin - 32.6(kg/g), Leucin - 48.9 (kg/g),Lysin-26.2(kg/g),Methionine-13.3(kg/g), Tryptophan - 8.5(kg/g)Valine-37.4(kg/g)].It also rich in [Protein-60-70%,Mineral Average mg 100g-1,Carbohydrate 19%,Calcium-700,Lipids- 6%,Iron-100,Minerals-8%,Chromium-0.28,Water -7% ,Potassium-1400,Phosphours-800,Zinc-3,Magnisium-400. Amino acid in Spirulina powder (mercus Rohrer 1994).]

Superfoods Spirulina: Unlike blue-green algae harvested from uncontrolled lakes, Spirulina is cultivated under carefully controlled conditions. The freshly harvested algae is rapidly dewatered before drying it in specially designed drying chambers, which ensures that heat sensitive vitamins and carotenoids are well preserved.

How to use Spirulina

Spirulina has a unique, strong, salty taste, which some find unpleasant. The powder is therefore best mixed with your favorite smoothie or fresh juice. Start with ½ a teaspoon and gradually build up to 1-2 teaspoon of Spirulina powder daily. Tablets can be taken at any time during the day with water. A recommended minimum dose is 3 g and should be eaten daily for maintenance.

Way to increase hemoglobin levels

Tips to Increase Hemoglobin Levels

As mentioned above, the underlying cause and the degree of variation in hemoglobin count may not be

the same for all. So the method used for rectifying the condition may also vary from one person to another. If there is no underlying disease, a nutritious diet, along with supplements, will suffice. Otherwise, the underlying medical condition has to be treated. If the count is too low, blood transfusion may be recommended. Such circumstances usually arise in the case of severe blood loss due to trauma, surgery or certain diseases.

Food Sources and Supplements

In case of iron deficiency anemia, the doctor may advise you to take iron supplements and include iron-rich foods in the diet. It is believed that iron in food from animal sources. Vitamins B6, B12 and folic acid are also found beneficial for increasing hemoglobin levels.

Avoid iron blockers

While taking iron supplements and iron-rich food, you have to avoid intake of certain food items, which affect the body’s ability to absorb iron. These iron-blockers include tea, coffee, food rich in fiber, foods with high levels of calcium (like milk, cheese ,chocolate, caffeinated drinks) and some medicines with phosphates and antacids.

Avoid foods with gluten

Those with celiac disease must avoid foods with gluten, as it can interfere with absorption of nutrients, like iron. So avoid wheat and wheat products and other foods that contain gluten to avoid iron deficiency and result and low hemoglobin levels.

Increase intake of vitamin C

It will be more beneficial if you take vitamin C (or food rich in vitamin C), along with iron supplements. Vitamin C is useful for increased absorption of iron in the body, which in turn enhances hemoglobin levels.

The phosphorous makes Spirulina useful for the tooth rematerializing regimen, and it is best taken

with an Omega-3 source like fermented cod liver oil. It's anti-inflammatory properties have been helpful to some of the people with joint pain or other types of inflammation.

My present study is to analysis the effect of spirulina in take among adolescent girls and women by performing complete blood count for a period of 3 month randomly.

Materials and Methods

Cultivation of spirulina by tank method:

Construction of tank

A flat site selected not surrounded by drainage water has been selected to construct tank in order to avoid microbial contamination.Measurement and requirements to construct tank (1000 ml capacity):Length of the tank-10feet,Width of the tank-5 feet,Height of the tank-1.5 feet,2×2 reefer 10 feet -4 no's,2×2 reefer 5 feet - 4 no's,2×2 reefer 11/2 feet-14 no's,Canvas sheet 19×9 feet,Tarpaulin sheet-13×10 feet,Bolt, nut, washer. The frames supporting the transparent covering of the pond is made of 6-8 mm diameter bars of bamboo

Process of spirulina cultivation:

Starter culture – *Arthrospira platensis*
Chemicals + Startert culture are poured in tank

Agitation
proper temperature and PH Maintained

↓
Harvesting

Filtering
↓
by using machine
Pressing

Grinding

storage in dark place at 20 -25⁰C and should not freeze.

Addition of medium to the tank

1.Potable water with low calcium content (less than 100 mg/liter) and slightly salty water (4-5 g/liter of Nacl) was selected.2. About 1000 its of water was added to the tank to a height of about one to two feet.Minerals are than added to the water such as Sodium bi carbonate-8grams, Sodium chloride-5 grams,Urea -0.2 gram,Potassium Sulphate-0.5 gram,Magnesium Sulphate -0.16 gram,Phosphoric acid - 0.052 ml,Ferrous Sulphate-0.05 ml,

Addition of mother inoculum

1 Kg of Spirulina (*Arthrospira platensis*) mother culture obtained from “Lakshmi Spirulina farm and Spirulina production technology, Thirubhuvani village, Puducherry” was added to the tank in fresh condition.

Parameters to be maintained for growth of spirulina

Depth, Temperature, Growth, Density, pH.

Harvesting

The culture concentration on the secchi disk goes below 2-3 cm indicates that Spirulina is ready for harvesting.Requirements for harvesting:A blue color net (1×1 meter),Mesh cloth (450 holes/1cm),Filter cloth,Basin,Bucket and Spatula.Early morning to harvest the Spirulina. Hands are cleanly washed with soap water or detergent. A blue color net with a screen printing cloth was placed across the tank. With another net well grown Spirulina gets filtered at bottom of the cloth, the dust and unwanted material gets settled on the top of the first layer. The collected Spirulina taken with help of spatula and washed with normal water to remove chemical materials.

Pressing

The drained Spirulina is then wrapped in clean muslin cloth and pressed under a weight of 50kg to further drain the moisture. It was then put in small

machines and squeezed in the form of noodle on a dry, clean cloth under the sun to dry for 4-5 hours.

Grinding

Hand, mouth and head should be covered with gloves, the dried Spirulina was ground using grinding machine and kept in a closed room free of contamination.

Storage of Spirulina powder

Spirulina powder packed in an air tight container and stored in a dark place at 20-25 °C and should not be freeze that favors lysis and death of cells

Spirulina as a haemtonic:

Subjects

Subjects were recruited from our college premises and interested individuals were initially screened for eligible criteria. Subjects were eligible if they had age <30 years and a diagnosis of anemia (HGB level,<12g/dl) during previous 3 months. Eligible subjects were undergoes a clinical evaluation for further health history assessment. Exclusion criteria include: any underlying neiplasia or immunological disease;the use of oral steroids or other immunosuppressive agents: ongoing prolonged use of Spirulina;food faddism or other non-traditional diet; known history of Spirulina allergy; chronic renal failure;chronic inflammatory diseases taking daily doses pf anti inflammatory drugs for longer than 4 weeks at the time of enrollment;and reduced physical activity. Enrolled subjects completed a food frequency questionnaire (FFQ) to provide information on their regular eating habits. The FFQ was developed by Nutritionquest (Berkeley, UK,USA) and has been extensively used and validated for research purposes to include nutrients in their diet. Once included in the study the subjects were instructed to take 2 tablets of 500mg Spirulina per day for a 12-week period. Blood sample were collected at 0,6 and 12weeks.

CBC variables

Blood samples collected from subjects were tested for using routine laboratory methods and gathered

data included HGB,Hematocrit (HCT), Mean Corpuscular HGB Concentration (MCHC), Mean corpuscular HGB (MCH), Mean Corpuscular Volume (MCV), and White Blood Cells (WBCs).

Results and Discussion

Spirulina was cultivated by open tank method. A mat form of Spirulina was harvested and various microbiological and biochemical tests were performed.

Identification of *Anthrospira platensis*.

Physical analysis

Color - Dark green color
Odour and taste - Mild like seaweed
Appearance - Fine

Microscopic analysis

S.No	TEST	Result
1	Gram's	Rod (negative)
2	Motility	Motile

Bacteriological examination

Standard plate count	47 CFU/0.1 ml
Sabouraud Dextrose agar	No growth of mold & yeast
Eosin Methylene Blue agar	No growth of coliforms
Salmonella-Shigella agar	No growth of Salmonella
Nutrient agar	No growth of Staphylococcus

Molecular weight with that of a high and low molecular comparision of cultivated spirulina plasmid DNA weight standards.

From comparison of the isolated plasmid DNA of Spirulina with that of standard *E.coli* plasmid of high molecular weight and standard Spirulina's plasmid DNA of low molecular weight, it was

Figure 1. Estimation of Hemoglobin Levels

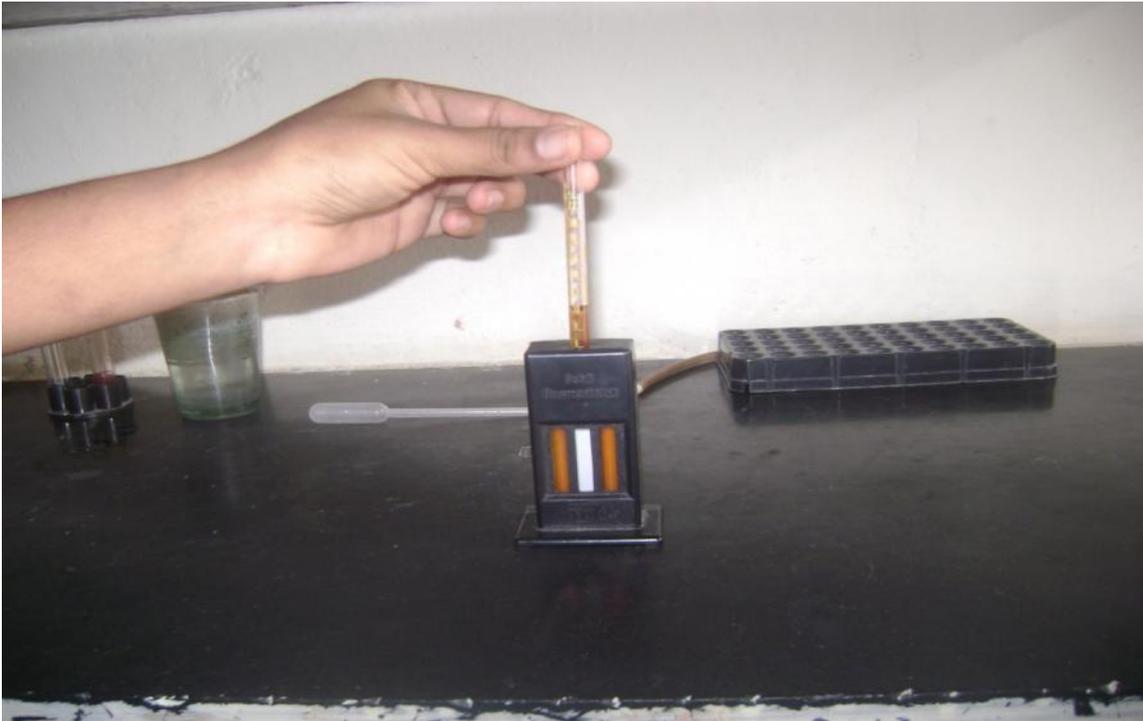


Figure 2. Cultivation of spirulina by tank method



found that our Spirulina's plasmid DNA's molecular weight was more over similar to that of standard low molecular weight, so it is found that Spirulina can be easily digested by human being due to its low molecular weight.

CBC Evaluation

CBC variables at baseline, week 6 and week 12 are as follows:

Raw values of CBC variables of study participants at the three time points (weeks 0,6,12).

Difference in the values of each variable between baseline, week 6 and week 12 were studied. We observed a significant increase in MCH during supplementation. Over ¾ th of the subjects had increased values of HCT, MCV and MCHC after 12 weeks an increase in WBCs and haemoglobin level was observed in 80% of the subjects.

Similar results were obtained during 2011, when Carlo Selmi, Patrick SC-Leung enrolled 40 volunteers of both sexes with an age of 50 years or both sexes with an age of 50 years or older who had no history of major chronic diseases. Participants took a Spirulina supplementation for 12 week and were administered comprehensive directly questionnaires to determine their nutritional regimen during the study. Complete Cell Count (CCC), as a sign of immune function, were determined at baseline and weeks 6 and 12 of supplementation.

Conclusion

Spirulina is the immortal descendent of the first photosynthetic life form. Beginning from 3.5 million year ago, blue-green algae created our oxygen atmosphere so that other life could evolve. Since then, algae have helped regulate our planet's biosphere.

Algae are two thirds of the earth's biomass. Thousands of algal species covering the earth are now being identified and can be used as food, pharmaceuticals, biochemical and fertilizers. Algae

represent one of the solutions we need to produce food while restoring our planet.

Scientists in the USA, India, Japan, China, Russia and other countries are studying this remarkable food (Spirulina) to unlock its potential. More research is need to determine its usefulness against AIDS and other killer diseases. However, it is clear this safe and considering the optimal safety profile of Spirulina, we encourage the design of larger clinical studies with solid endpoints and sufficiently long follow-up with appropriate randomization.

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