Clinical Chemistry Studies in Egyptian Patients with Hyperlipidemia

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Citation

Abstract
The present study was conducted to evaluate the efficiency of natural Spirulina preparation in decrease of hyperlipidemia. 20 Egyptian patients with history of hyperlipidemia, aged from 30 to 60 years were subjected in this study. Volunteers were supplied with dried Spirulina (4gm/day) by oral uptake. Throughout zero time (without treatment) and after 7, 14 and 21 days of treatment with Spirulina, serum total cholesterol, Triglycerides, High density lipoprotein (HDL), Low density lipoprotein (LDL), Alkaline Phosphatase, Aspartate Aminotransferase (SGOT), Alanine Aminotransferase (SGPT), Albumin, Total bilirubin and Direct bilirubin were assessed. This study proved that Spirulina has effect to decrease lipid profile tests and liver function tests in hyperlipidemia patients.

1. Introduction

The microscopic, filamentous prokaryote, Arthrospira (Spirulina), has been the subject of intense investigation mainly owing to its use as food, feed, dietary supplement, and functional food. Its recorded historical use as food spans centuries and it has been commercialized as food for the past 30 years. Though there are numerous studies on its mass production outdoors, these are mainly from studies involving small experimental outdoor ponds. There is therefore only limited information from large scale commercial facilities that produce Spirulina in large outdoor ponds. Small-scale production in experimental ponds is limited in its scope since it does not deal with effects of harvesting and recycling of nutrients on a continuous basis that affect both the yield and quality of the product [1].

Lipids, such as cholesterol and triglycerides, are insoluble in plasma and circulating lipid is carried in lipoproteins that transport the lipid to various tissues for energy utilization, lipid deposition, steroid hormone production, and bile acid formation. The lipoprotein consists of esterified and unesterified cholesterol, triglycerides, and phospholipids, and protein. The protein components of the lipoprotein are known as apolipoproteins or apoproteins [2].

One definition of hyperlipidemia is serum total cholesterol, LDL-cholesterol, triglyceride, concentrations above the ninetieth percentile, or HDL-cholesterol below the tenth percentile for the general population [3].

Abnormal lipoprotein metabolism is a major predisposing factor to atherosclerosis. It is estimated, for example, that a dyslipidemia is present in over 70 percent of patients
with premature chronic heart disease (CHD) [4].

Heart disease mortality has declined steadily since the 1960s, but CHD remains the leading cause of death for both men and women of all races and ethnicities in the United States. A large proportion of elderly individuals will suffer from CHD. In men over the age of 65, for example, nearly one-half of all deaths are attributed to CHD, compared to less than 25% for all cancers and less than 2% for all infections. An even higher proportion of deaths are due to CHD in older women (56%), with less than 20 percent due to cancer. The incidence is highest in patients with premature CHD, which is defined as clinically evident disease occurring before 55 years of age in men and before 65 years in women. In this setting, the prevalence of hyperlipidemia is as high as 80-88% compared to approximately 40-48% in age-matched controls without coronary disease [5]. The mean serum total cholesterol concentration of the adult United States population showed a small decline in 1999 to 2002 compared with that found in 1988 to 1994. Over the same periods, the percentage of US adults with a total cholesterol level of 240 mg/dL (6.22 mmol/L) or higher declined from 20-17% [5].

A variety of factors, often acting in concert, are associated with an increased risk for atherosclerotic plaques in coronary arteries and other arterial beds. Hypercholesterolemia is one of the major modifiable risk factors for CHD, even in those over the age of 65 [6].

Causes of Hyperlipidemia are classified to:

1. Primary causes of hyperlipidemias are associated with [7]:
   a) Overproduction of lipoproteins.
   b) Abnormality in the lipoprotein receptor.
2. Secondary causes of hyperlipidemias are Type 2 diabetes mellitus [8]:
   a) Cholestatic liver diseases.
   b) Hypothroidism.
   c) Cigarette smoking.
   d) Obesity.
   e) Drugs.

The major classes of hyperlipidemia are classified according to the Fredrickson phenotype [9]. A variety of defects; some are familial and can produce these disorders

1) Fredrickson phenotype I: serum concentration of chylomicrons elevated; triglycerides concentrations are elevated to >99th percentile.
2) Fredrickson phenotype IIa: serum concentration of LDL cholesterol elevated; the total cholesterol concentration is >90th percentile. Concentrations of triglyceride and/or apolipoprotein B may also be ≥ 90th percentile.
3) Fredrickson phenotype IIb: serum concentrations of LDL and VLDL cholesterol elevated; total cholesterol and/or triglycerides may be ≥ 90th percentile and apolipoprotein B ≥ 90th percentile.
4) Fredrickson phenotype III: serum concentration of VLDL remnants and chylomicrons elevated; total cholesterol and triglycerides >90th percentile.
5) Fredrickson phenotype IV: serum concentrations of VLDL elevated; total cholesterol may be >90th percentile and may also see triglyceride concentrations >90th percentile or low HDL.
6) Fredrickson phenotype V: elevated serum concentrations of chylomicrons and VLDL; triglycerides >99th percentile.

Because hyperlipidemia usually doesn't cause symptoms, you will need a blood test for a diagnosis. The National Cholesterol Education Program recommends that people get this test every 5 years after age 20. The blood test will show the levels of different lipids in blood. Most blood tests measure levels of LDL, HDL, total cholesterol (LDL plus HDL), and triglycerides. According to [10], to have a low risk of heart disease, your desirable lipid levels are

a) LDL less than 130 mg/dL
b) HDL greater than 40 mg/dL (men) or 50 mg/dL (women)
c) Total cholesterol less than 200 mg/dL
d) Triglycerides less than 200 mg/dL.

The major effect by which Spirulina reduces the level of fat in the liver is by reducing triacylglycerol levels in the serum and the liver. Having received SM, female mice exhibited reduced liver triacylglycerol and a significant decline (p <.05) in serum triacylglycerol. Male mice, however, exhibited a significant decrease (p <.05) in the triacylglycerol level in the liver rather than in the serum. Reduced triacylglycerol accumulation relieves the formation of fatty liver. The hypotriacylglycemic effect of Spirulina may help to reduce liver total lipid and thereby lower the risk of hepatic steatosis. Gonadectomized female animals reportedly are more likely to develop diabetes because of the effect of female sex steroids on glucose metabolism [11].

The hypcholesterolemic effect of Spirulina in humans has been reported [12]. Reduced serum cholesterol (4.5%), triacylglycerol and LDL were observed when Spirulina (4.2 g/day) was added for 8 weeks to the diet of thirty Japanese males with high cholesterol, mild hypertension, and hyperlipidemia. Serum cholesterol returned to its initial level if the intake of Spirulina was discontinued after 4 weeks. In addition, the hypcholesterolemic effect was greater in men with a higher cholesterol diet [13].

The effects of Spirulina orally supplied (4.5 g/day, for 6 weeks) to a sample of 36 subjects (16 men and 20 women, with ages between 18-65 years) on serum lipids, glucose, aminotransferases and blood pressure showed that there were no significant changes in the values of glucose and aspartate aminotransferase (AST), but significant differences in triacylglycerol (TAG), total cholesterol (TC), and cholesterol associated to high density lipoprotein (HDL-C). In addition, significant reductions were found comparing initial and final systolic (SYST-P) and diastolic (DIAST-P) blood pressure in both male and female, concluded that Spirulina maxima
showed a hypolipidemic effect [14].

2. Materials and Methods

2.1. Application on Human Volunteers

This study was done according to guidelines of Egyptian ministry of Health and Population decree 95/year 2005 for medical research, good clinical practice, Declaration of Helsinki and World Health Organization Guidelines [15]. Spirulina was approved by the FDA (Food Drug Administration) by the issuance of a GRAS (generally recognized as safe) certificate. The FDA has stated that Spirulina can be legally marketed as a food or food supplement without risk to human health [16].

Follow up of 20 Egyptian hyperlipidemia patients, aged from 30 to 60 years was performed, the laboratory tests were performed at zero time (without treatment) and after 7, 14 and 21 days of treatment with Spirulina. Volunteers were supplied with dried Spirulina (4gm/day) by oral uptake

2.2. Laboratory Equipments

1. Micopette pipettes, Dragon Lab Company, China.
2. Prietest Eco Automatic Biochemistry Analyzer, Robonik Company, India
3. Centrifuge Model 800, Xiangshui Fada Medical Apparatus factory, China.
5. Reagent used in detection of SGPT and SGOT, Bilirubin, Alkaline phosphatase and Albumin
6. Reagent used in detection of Serum total cholesterol, Triglycerides, High density lipoprotein (HDL) and Low density lipoprotein (LDL)

2.3. Blood Sampling and Serum Preparation

Blood samples were taken from Egyptian hyperlipidemia patients from Tanta University Hospital. All samples were directed for the following tests:

2.3.1. Determination of Serum Cholesterol Level

Total serum cholesterol level was determined by an enzymatic colorimetric method using a diagnostic kit supplied by Stanbio; USA [17].

Principle:
The method depends on the following reactions:

a) Cholesterol esters → free cholesterol + fatty acids
b) Cholesterol + O₂ → 4-cholesten - 3-one +H₂O₂
c) H₂O₂ + Phenol + 4-aminoantipyrine → red quinine + 4H₂O₂

The density of red color product is measured spectrophotometrically.

Reagent:
(A) Reagent:
1. Buffer and stabilizers
2. Phenol 25 mmol/L
3. aminoantipyrine 0.25 mmol/L
4. Cholesterol esterase >0.15 U/L
5. Cholesterol oxidase >0.1 U/L
6. Peroxidase >5.0 U/L
(B) Standard cholesterol 200mg/dl

Procedure:
1. Sample, standard and reagent are added to labeled dry test tube as following:

<table>
<thead>
<tr>
<th>Working reagent</th>
<th>Blank</th>
<th>Standard</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Working reagent</td>
<td>1 ml</td>
<td>1 ml</td>
<td>1 ml</td>
</tr>
<tr>
<td>Standard</td>
<td>-</td>
<td>10 µl</td>
<td>-</td>
</tr>
<tr>
<td>Sample</td>
<td>-</td>
<td>-</td>
<td>10 µl</td>
</tr>
</tbody>
</table>

2. The tubes are mixed thoroughly, and then incubated at 37°C for 5 minutes.
3. The optical densities (OD) of both sample and standard are measured against reagent blank at λ 500 nm.

Calculation:

Total cholesterol concentration (mg/dl) = [OD sample /OD standard] × n

Where (n) Concentration of standard cholesterol = 200 mg/dl.

2.3.2. Determination of Triglycerides

Principle:

Sample triglycerides incubated with lipoprotein lipase, liberate glycerol and free fatty acids. Glycerol is converted to glycerol-3-phosphate and adenosine-5-Diphosphate by glycerol kinase and ATP. Glycerol-3-phosphate is then converted by glycerol -3- oxidase to dihydroxyacetone phosphate and hydrogen peroxide. In the last reaction, hydrogen peroxide reacts with 4-aminophenazone and p-chlorophenol in presence of peroxidase to give a red colored dye:

a) Triglycerides + H₂O → Glycerol + free fatty acids
b) Glycerol + ATP → Glycerokinase → G3P + ADP
c) G3P + O₂ → DAP + H₂O₂
d) H₂O₂ + 4-AP + p - chlorophenol → Quinone + H₂O₂

The intensity of the color formed is proportional to the triglycerides concentration in the sample [18].
Table 2. Reagents of Triglycerides.

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Component</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffer</td>
<td>GOOD pH 7.5 50 mmol/L</td>
</tr>
<tr>
<td></td>
<td>p-Chlorophenol 2 mmol/L</td>
</tr>
<tr>
<td></td>
<td>Lipoprotein lipase (LPL) 150000 U/L</td>
</tr>
<tr>
<td></td>
<td>Glycerokinase (GK) 500 U/L</td>
</tr>
<tr>
<td>Reagent</td>
<td>Glycerol-3-oxidase (GPO) 2500 U/L</td>
</tr>
<tr>
<td>Enzymes</td>
<td>Peroxidase (POD) 440 U/L</td>
</tr>
<tr>
<td></td>
<td>4-Aminophenazone (4-AP) 0.1 mmol/L</td>
</tr>
<tr>
<td></td>
<td>ATP 0.1 mmol/L</td>
</tr>
<tr>
<td>TRIGLYCERIDES CAL</td>
<td>Triglycerides aqueous primary standard 200 mg/dL</td>
</tr>
</tbody>
</table>

Procedure:

1. Assay conditions:
   - Wavelength: 505 nm (490-550)
   - Temperature 37°C / 15-25°C
2. Adjust the instrument to zero with distilled water.
3. Pipette into cuvette 1.0 ml of blank 1.0 ml of standard and 1 ml of sample then pour 10 µl of standard then added 10 ul of sample.
4. Mix and then incubate for 5 minutes at 37°C.
5. Read the absorbance of sample and standard against blank.

Calculation:

\[ \text{Triglycerides} = \left(\frac{A_{\text{Sample}}}{A_{\text{Standard}}} \right) \times 200 \] (Standard conc.) = mg/dL

2.3.3. Determination of High Density Lipoprotein (HDL)

Principles of reaction:

This technique uses a separation method based on the selective precipitation of apolipoprotein B-containing lipoproteins (Very Light Lipoprotein, Low Density Lipoprotein and a) Lpa) by phosphotungsic acid/MgCl₂, sedimentation of the precipitant by centrifugation, and subsequent enzymatic analysis of high density lipoproteins (HDL) as residual cholesterol remaining in the clear supernatant [19].

Reagent composition:

a) Reagent:
   - Precipitating reagent. Phosphotungstic acid 0.63 mmol/L,
   - Magnesium chloride 25 mmol/L, sterilizers.

b) Standard: HDL-Cholesterol standard mg/dL

Procedure:

I Precipitation:
1. Bring reagents and samples to room temperature.
2. Pipette into labelled centrifuge tubes:
3. Vortex and allow to stand for 10 minutes at room temperature.
4. Centrifuge for 10 minutes at 4000 r.p.m or 2 minutes at 12000 r.p.m
5. Separate of the clear supernatant within 2 hours.

II Colorimetry:

1. Bring the cholesterol MR mono-reagent and the cholesterol standard (50 mg/dL) of the kit to room temperature.
2. Pipette into labelled centrifuge tubes.

3. Mix and lit the tubes stand for 10 minutes at room temperature or 5 minutes at 37°C.
4. Read the absorbance (A) of the supernatant and the standard at 550 nm against the reagent blank.

The color is stable for at least 30 minutes protected from light.

Calculation:

\[ \frac{A_{\text{supernatant}}}{A_{\text{standard}}} \times C_{\text{standard}} = \text{mg/dL HDL-Cholesterol} \]

If results are to be expressed as SI units apply: mg/DL × 0.0259 = mmol/L

2.3.4. Determination of Low Density Lipoprotein (LDL)

Low Density Lipoprotein was determined for human and experimental animals by using the following equation [20]:

\[ \left[ \text{Triglycride/5} \right] + \left[ \text{HDL – cholesterol} \right] = \text{mg/dl} \]

2.3.5. Bilirubin: Total and Direct

Principles of reaction:

Most chemical methods for bilirubin determination are based on the reaction between diazotized sulfanilic acid and bilirubin in the presence or absence of an organic solvent to distinguish free from conjugated bilirubin on a differential solubility basis. In aqueous solution only conjugated or direct bilirubin will react. To measure total bilirubin, it is necessary to add an accelerator or solvent [21].

Reagents:

a) Reagent 1: Sulfanilic acid 31 mmol/I
   - HCI 0.2 N
b) Reagent 2: Sodium nitrite 28 mmol/I
c) Reagent 3: Caffeine 0.28 mol/l
   - Sodium benzoate 0.55 mol/
d) Reagent 4: Tartrate 0.99 mol/I
   - NaOH 2.0 N

Wave length adjustment of F- T- 2 Spectrophotometer (Italy):

Total Bilirubin 578 nm (560-600 nm)

Direct Bilirubin 546 nm (530-555 nm) Optical path 1 cm

Zero adjustment Specimen blank

Determination of total bilirubin:

0.2 ml from reagent 1 poured in two different tubes; one for sample and one for blank). For specimen, one drop from reagent 2 (100 ml) was added, 1 ml from reagent 3 was added for test and samples under examination from serum and 0.2 ml was added to test and blank.

Table 3. Reagent in precipitation Step in High Density lipoprotein Determination.

<table>
<thead>
<tr>
<th>Sample or Standard 0.2 mL</th>
<th>Precipitating reagent 0.4 mL</th>
<th>Ratio Sample / Reagent = 1/2</th>
<th>Dil. Factor = 3</th>
</tr>
</thead>
</table>

Table 4. Reagent in Colorimetric Step in High Density Lipoprotein Determination.

<table>
<thead>
<tr>
<th>Tubes</th>
<th>Blank</th>
<th>Sample supernate</th>
<th>Standard supernate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monoreagent</td>
<td>1.0 ml</td>
<td>1.0 ml</td>
<td>1.0 ml</td>
</tr>
<tr>
<td>Supernats</td>
<td>-</td>
<td>50 µL</td>
<td>-</td>
</tr>
<tr>
<td>Standard</td>
<td>-</td>
<td>-</td>
<td>50 µL</td>
</tr>
</tbody>
</table>
The later mixtures were incubated at 20-25°C for 10 minutes. One ml from reagent 4 was added to test and standard. The mixture incubated for 5 min at 20-25°C. The absorbances for samples or specimen against blank was reading at 578 nm. Total bilirubin was calculated by following equation:

\[ \text{Total bilirubin} = \text{As} \times 10.8 \]

Determination of direct bilirubin:

To measure of direct bilirubin; about 0.2 ml from reagent 1 in blank test tube and different tubes for different specimen were prepared. A drop from reagent 2 was added to specimen only. 2 ml from NaCL (0.9%) solution was added to test and blank, Serum (0.2 ml) under examination was added to Blank and specimen tubes. Direct bilirubin was calculated by following Equation:

\[ \text{Direct bilirubin} = \text{As} \times 14.4 \]

### Table 5. Steps of Determination of Serum Total Bilirubin.

<table>
<thead>
<tr>
<th>Specimen blank</th>
<th>Specimen</th>
</tr>
</thead>
<tbody>
<tr>
<td>R₁</td>
<td>0.2 ml</td>
</tr>
<tr>
<td>R₂</td>
<td>1 drop</td>
</tr>
<tr>
<td>R₃</td>
<td>1.0 ml</td>
</tr>
<tr>
<td>Specimen</td>
<td>0.2 ml</td>
</tr>
<tr>
<td>Mix, and incubate for 10 minutes at 20 - 25°C. Add</td>
<td></td>
</tr>
<tr>
<td>R₄</td>
<td>1.0 ml</td>
</tr>
</tbody>
</table>

Mix, and incubate for 5 minutes at 20-25°C. Read absorbance of specimen against specimen blank. The color intensity is stable for 30 min.

### Table 6. Steps of Determination of Serum Direct Bilirubin.

<table>
<thead>
<tr>
<th>Reagents</th>
<th>Specimen blank</th>
<th>Specimen</th>
</tr>
</thead>
<tbody>
<tr>
<td>R₁</td>
<td>0.2 ml</td>
<td>0.2 ml</td>
</tr>
<tr>
<td>NaCl (0.9%)</td>
<td>2.0 ml</td>
<td>2.0 ml</td>
</tr>
<tr>
<td>Specimen</td>
<td>0.2 ml</td>
<td>0.2 ml</td>
</tr>
</tbody>
</table>

Mix and incubate for exactly 5 minutes at 20-25°C. Read absorbance of specimen against specimen blank.

The mixtures were incubated for 5 minutes at 20–25°C. Specimens were reading against blank at 580 nm.

### 2.3.6. Determination of Alanine Aminotransferase; SGPT (ALAT)

**Method:**

Kinetic method for the determination of ALAT activity is according to the recommendation of the Expert panel of the IFCC (International Federation of Clinical Chemistry), without Pyridoxalphosphate activation [20].

**Reaction principle:**

a) \[ 2\text{-oxoglutarate} + \text{L-aspartate} \rightleftharpoons \text{L-glutamate} + \text{oxaloacetate} \]

b) \[ \text{Pyruvate} + \text{NADH} + \text{H}^+ \rightleftharpoons \text{L-malate} + \text{NAD}^+ \]

### Table 7. Procedure of Determination of Alanine Aminotransferase.

<table>
<thead>
<tr>
<th>Pipette into cuvettes</th>
<th>37°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
<td>100 μl</td>
</tr>
<tr>
<td>Working reagent</td>
<td>1000 μl</td>
</tr>
</tbody>
</table>

Mix and then read the absorbance after 1 minute and at the same time start the stopwatch and then read the absorbance again exactly after 30, 60 and 120 seconds.

Calculation:

The rate of AS changes through 30, 60 and 120 seconds were calculated at 340 nm of wave length according to this equation:

\[ \text{At 340 nm} \]

\[ \text{Equation} = \Delta \text{AS} \times 1745 \]

### 2.3.7. Determination of Serum Aspartate Aminotransferase (SGOT)

**Method:**

It was revealed that the kinetic method for the determination of SGOT activity is according to the recommendations of the Expert Panel of the IFCC (International Federation of Clinical Chemistry), without pyridoxal phosphate activation [22].

**Reaction Principle:**

\[ \text{GOT} \]

\[ a) \ 2\text{-oxoglutarate} + \text{L-aspartate} \rightleftharpoons \text{L-glutamate} + \text{oxaloacetate} \]

\[ \text{MDH} \]

\[ b) \ \text{Oxaloacetate} + \text{NADH} + \text{H}^+ \rightleftharpoons \text{L-malate} + \text{NAD}^+ \]

### Table 8. Reagents Used In Determination of Serum Aspartate Aminotransferase.

<table>
<thead>
<tr>
<th>Buffer</th>
<th>Buffer / Enzyme reagent</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRIS buffer (ph 7.8)</td>
<td>100 mmol/l</td>
</tr>
<tr>
<td>L-aspartate</td>
<td>300 mmol/l</td>
</tr>
<tr>
<td>LDH</td>
<td>≥ 0.9 kU/l</td>
</tr>
<tr>
<td>MDH</td>
<td>≥ 0.6 kU/l</td>
</tr>
<tr>
<td>Substrate</td>
<td></td>
</tr>
<tr>
<td>2-oxoglutarate</td>
<td>60 mmol/l</td>
</tr>
<tr>
<td>NADH</td>
<td>0.9 mmol/l</td>
</tr>
</tbody>
</table>

The entire contents of substrate bottle were poured into bottle contained buffer. The working reagents stable for 4 weeks at 2-8°C

**Procedure:**

1. Pipette into cuvettes at 37°C 100 μl Sample and 1000 μl of working reagent.
2. Mix and then read the absorbance after 1 minute and at the same time start the stopwatch. Read the absorbance again exactly after 30, 60 and 120 seconds.

Calculation:

The rate of AS changes through 30, 60 and 120 seconds were calculated at 340 nm of wave length according to this equation:

\[ \text{At 340 nm} \]

\[ \text{Equation} = \Delta \text{AS} \times 1745 \]

### 2.3.8. Determination of Total Proteins

**Principle:**

Proteins give an intensive violet-blue complex with copper salts in an alkaline medium. Iodide is included as an antioxidant. The intensity of the color formed is proportional to the total protein concentration in the sample [23].
Table 9. Reagents used In Determination of Serum Total Protein.

<table>
<thead>
<tr>
<th>Total Protein Standard Reagent</th>
<th>Bovine albumin primary standard 6 g/dL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent 2 Biuret</td>
<td>Sodium potassium tartrate 15 mmol/L</td>
</tr>
<tr>
<td></td>
<td>Sodium iodide 100 mmol/L</td>
</tr>
<tr>
<td></td>
<td>Potassium iodide 5 mmol/L</td>
</tr>
<tr>
<td></td>
<td>Copper (H) sulphate 19 mmol/L</td>
</tr>
</tbody>
</table>

Procedure:
1. Prepare 3 cuvettes and then pour 1 ml of blank, standard and sample.
2. Pour 20 ul of standard and 20 ml of sample.
3. Mix and incubate for 5 minutes at room temperature.
4. Read the absorbance (A) of the samples and Standard, against the Blank. The colour is stable for at least 30 minutes.

Calculation:
Total protein in sample = \([A \text{ Sample} / A \text{ Standard}] \times 6.0\) (Standard conc.) = g/dL

2.3.9. Determination of Human Albumin

Method:
Bromocresol green forms with albumin in citrate buffer a colored complex. The absorbance of this complex is proportional to the albumin concentration in the sample [24].

Contents:
a) Working reagent:
   1. Citrate buffer (pH 4.2) 30 mmol/l
   2. Bromocresol green 260 µmol/l
b) Standard:
   1. Albumin 4 g/dl
   2. Sodium azide 0.095%

Procedure:
Ten ml from standard, collected serum samples were added to 1 ml from working reagent and the mixture was left at room temperature for 5 min. The absorbance of standard and serum sample was read at 578 nm against reagent blank [25].

Calculation:
Albumin concentrations of sample were estimated by using the following equation:

\[ C = 4 \times \Delta A_{\text{sample}} / \Delta A_{\text{STD}} \ [\text{g/dL}] \]

2.3.10. Determination of Alkaline Phosphatase

Principle:
Phenylphosphate is hydrolyzed in alkaline pH by the action of alkaline phosphatase into phenol and phosphate. The phenol liberated is measured in the presence of 4-aminoantipyrine and potassium Ferricyanide. The presence of sodium arsenate in the reagent stops the enzymatic reaction [26].

Table 10. Reagents used In Determination of Alkaline Phosphatase.

<table>
<thead>
<tr>
<th>Reagent (1) (Buffer substrate)</th>
<th>Disodium phenylphosphate 5 mM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent (2) (Standard)</td>
<td>Carbonate-bicarbonate buffer 50 mM</td>
</tr>
<tr>
<td>Reagent (3) (Blocking reagent)</td>
<td>Phenol equal to 20 Kind &amp; King U</td>
</tr>
<tr>
<td>Reagent (4) (Color reagent)</td>
<td>4 Aminoantipyrine 60 mM</td>
</tr>
<tr>
<td></td>
<td>Sodium arsenate 240 mM</td>
</tr>
<tr>
<td></td>
<td>Potassium Ferricyanide 150 mM</td>
</tr>
</tbody>
</table>

Procedure:
1. Adjust the Wave length 510 nm and Zero adjustment of Reagent blank.
2. Pipette into 4 clean dry test tubes:
   Sample 2 ml
   Serum Blank 2 ml
   Reagent Blank 2 ml
   Standard 2 ml
3. The mixture was incubated for 5 minutes at 37°C.
4. 50µL of serum were poured on sample and 50µL of Reagent (2) were added on standard tube.
5. The tube incubated for exactly 15 minutes at 37°C.
6. 0.5ml of Reagent (3) was poured on each tube, and mix well preferably vortex.
7. 0.5ml of Reagent (4) was added on each tube.
8. 50µL of serum was added on serum blank and 50µL of distilled water was added on Reagent Blank.
9. Mix and let stand for 10 minutes in the dark. Then measure the absorbance of serum sample (As), of serum blank (Asb) and Absorbance of standard (Astd) against Reagent blank at 510 nm.

Calculation:
\[ \text{ALP activity} = \frac{\text{absorbance of serum sample} - \text{absorbance of serum blank}}{\text{Absorbance of standard} \times 20 RU} \times \frac{\text{IU}}{L} \]

3. Results

Application on human; 20 randomly volunteers patients collected with previous history of hyperlipidemia and get the Spirulina treatment for 1, 2 and three weeks.

Table 11 and Figure 1 present the serum lipids profiles of 20 patients with history of hyperlipidemia at zero time (without treatment) and after 7, 14 and 21 days of treatment with Spirulina. Serum cholesterol level shows highly significant decrease after one, two and three weeks with Spirulina treatment by 15.12%, 23.9% and 28.44% respectively. Triglycerides level shows highly significant decrease by 10.6%, 20.5% and 31.6% after one, two and three weeks respectively with Spirulina treatment. High Density Lipoprotein level shows significant increase after one week of Spirulina treatment by 15.12%, 23.9% and 28.44% respectively. Triglycerides level shows highly significant decrease by 10.6%, 20.5% and 31.6% after one, two and three weeks respectively with Spirulina treatment. High Density Lipoprotein level shows significant increase after one week of Spirulina treatment by 7.5% significantly after two and three weeks of Spirulina treatment by 22% and 28% respectively. Low Density Lipoprotein level shows highly significant decrease after one, two and three weeks with Spirulina treatment by 19.9%, 32.2% and 37.7% respectively.
Table 11. Collective data of Serum lipids profile of 20 patients with history of hyperlipidemia at zero time (without treatment) and after (7, 14 and 21 days) of treatment with Spirulina.

<table>
<thead>
<tr>
<th>DAYS</th>
<th>Cholesterol</th>
<th>TRI</th>
<th>HDL</th>
<th>LDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>257.9±7.3*</td>
<td>155.2±20.7*</td>
<td>36.8±0.6*</td>
<td>190.4±7.6*</td>
</tr>
<tr>
<td>7</td>
<td>218.9±6.3***</td>
<td>138.7±20.6***</td>
<td>39.5±0.7**</td>
<td>154.2±6.3***</td>
</tr>
<tr>
<td>14</td>
<td>196.2±7.7***</td>
<td>123.4±20.6***</td>
<td>44.9±2.6***</td>
<td>129.3±5.1***</td>
</tr>
<tr>
<td>21</td>
<td>184.5±7.09***</td>
<td>106.1±16.4***</td>
<td>47.1±1.3***</td>
<td>118.6±5.6***</td>
</tr>
</tbody>
</table>

NB.: Each value is the mean ± standard error of the mean.

*** Highly significant at P≤0.001, ** Significant at P≤0.01, * Low significant at P≤0.05.

Data present in Table 12 and Figure 2 showed liver function of 20 patients with history of hyperlipidemia at zero time (without treatment) and after 7, 14 and 21 days of treatment with Spirulina; total bilirubin shows no change after one week of Spirulina treatment and after two and three weeks there is decrease in total bilirubin by 0.1% in comparison with control. Direct bilirubin shows decreases after one, two and three weeks of Spirulina treatment by 0.9%, respectively. Protein concentration shows no change after one week of Spirulina treatment but after two and three weeks it increased by 0.8% and 2.4% respectively. Albumin level shows no significant change after one, two and three weeks of Spirulina treatment there were a significant decrease 0.9%. Alanine Aminotransferase (SGPT) shows increases after one week of Spirulina treatment by 5.3% but after two and three weeks there is significant decrease by 12.6% and 16% respectively. Aspartate Aminotransferase (SGOT) shows increase after one week of Spirulina treatment by 1%, after two weeks there is decrease by 2.5% and after three weeks there is increase again by 3.2%. Alkaline Phosphatase level shows no changes after one week of Spirulina treatment but after two and three weeks it decreased by 1% and by 8.1% respectively.

Figure 2. Collective data of liver function of 20 patients with history of hyperlipidemia at zero time (without treatment) and after 7, 14 and 21 days of treatment with Spirulina.
blood pressure. The volunteers did not modify their dietary habits or lifestyle during the whole experimental period.

From each subject, a sample of blood was drawn in fasting condition at zero time (without treatment) and after 7, 14 and 21 days of treatment with Spirulina. Collective data of Serum lipids profile changes on 20 patients with history of hyperlipidemia at zero time (without treatment) and after 7, 14 and 21 days of treatment with Spirulina demonstrated that there was a highly significant decrease in total cholesterol, triglyceride and LDL with highly significant increase in HDL.

These results are in agreement with a study of thirty healthy men with high cholesterol, mild hypertension and hyperlipidemia showed lower serum cholesterol, triglyceride and LDL (undesirable fat) levels after eating Spirulina for eight weeks. These men did not change their diet, except adding Spirulina. No adverse effects were noted. Spirulina did lower serum cholesterol and was likely to have a favorable effect on alleviating heart disease since the atherogenic indices, TC: HDL-C, were observed. The calculated values for cholesterol associated to high density lipoprotein (HDL-C), aspartate aminotransferase (AST) and Body Mass Index (BMI). There were significant differences in TAG, TC, and HDL-C, were observed. The calculated values for cholesterol associated to low density lipoprotein (LDL-C) were significantly reduced by the Spirulina treatment [14]

4. Discussion

In the present study collective data of liver function test changes on 20 patients with history of hyperlipidemia at zero time (without treatment) and after 7, 14 and 21 days of treatment with Spirulina revealed that there were a significant decrease in SGPT and SGOT after 7 and 14 days of treatment with Spirulina. Collective data of Serum lipids profile changes on 20 patients with history of hyperlipidemia at zero time (without treatment) and after 7, 14 and 21 days of treatment with Spirulina demonstrated that there were a highly significant decrease in total cholesterol, triglyceride and LDL with highly significant increase in HDL.

In the same direction a results of a study aimed to evaluate the hypoglycemnic and hypolipidemic role of Spirulina. Twenty-five patients with type 2 diabetes mellitus were randomly assigned to receive Spirulina (study group) or to form the control group. With regard to lipids, triglyceride levels were significantly lowered. Total cholesterol (TC) and its fraction, low-density lipoprotein cholesterol (LDL-C), exhibited a fall coupled with a marginal increase in the level of high-density lipoprotein cholesterol (HDL-C). As a result, a significant reduction in the atherogenic indices, TC: HDL-C and LDL-C: HDL-C, was observed [28].

In agreement with these results, a study made on 23 patients showed that TC significantly decreased by 116.33 mg/dl, LDL-C by 94.14 mg/dl, and triglycerides by 67.72 mg/dl and helped reduce the increased levels of lipids in patients with hyperlipidemic nephrotic syndrome [29].

In the same way a study evaluate the effects of Spirulina maxima orally supplied (4.5 g/day, for 6 weeks) to a sample of 36 subjects (16 men and 20 women, with ages between 18-65 years) on serum lipids, glucose, aminotransferases and on blood pressure. The volunteers did not modify their dietary habits or lifestyle during the whole experimental period. From each subject, a sample of blood was drawn in fasting state of 12 hours to determine the plasma concentrations of glucose, triacylglycerols (TAG), total cholesterol (TC), and cholesterol associated to high density lipoprotein (HDL-C), aspartate aminotransferase (AST) and Body Mass Index (BMI). There were significant differences in TAG, TC, and HDL-C, were observed. The calculated values for cholesterol associated to low density lipoprotein (LDL-C) were significantly reduced by the Spirulina treatment [14].

5. Conclusion

The thrust of this study to measure serum total cholesterol, Triglycerides, High density lipoprotein (HDL), Low density lipoprotein (LDL), Alkaline Phosphatase, Aspartate Aminotransferase (SGOT), Alanine Aminotransferase (SGPT), Albumin, Total bilirubin and Direct bilirubin were assessed. This study proved that Spirulina has effect to decrease lipid profile tests and liver function tests in hyperlipidemia patients.

References


Table 12. Collective data of liver function of 20 patients with history of hyperlipidemia at zero time (without treatment) and after 7, 14 and 21 days of treatment with Spirulina.

<table>
<thead>
<tr>
<th>DAYS</th>
<th>Alk (mg/dL)</th>
<th>Albumin (g/dL)</th>
<th>Protein (g/dL)</th>
<th>SGOT (U/L)</th>
<th>SGPT (U/L)</th>
<th>Bil D (mg/dL)</th>
<th>Bil T (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>11.2±1.2*</td>
<td>4.08±0.1*</td>
<td>7.06±0.1*</td>
<td>47.7±11.4*</td>
<td>51.4±9.8*</td>
<td>0.15±0.2*</td>
<td>0.73±0.4*</td>
</tr>
<tr>
<td>7</td>
<td>11.2±1.2*</td>
<td>4.08±0.0*</td>
<td>7.06±3.2*</td>
<td>48.1±7.9*</td>
<td>54.1±8.6*</td>
<td>0.14±0.1**</td>
<td>0.73±0.4*</td>
</tr>
<tr>
<td>14</td>
<td>11.1±1.1*</td>
<td>4.1±0.07*</td>
<td>7.1±0.1*</td>
<td>46.5±7.9*</td>
<td>44.9±5.8*</td>
<td>0.13±0.1**</td>
<td>0.72±0.3*</td>
</tr>
<tr>
<td>21</td>
<td>10.2±1.09*</td>
<td>4.1±0.1*</td>
<td>7.2±0.1*</td>
<td>49.2±6.7*</td>
<td>38.06±4.8**</td>
<td>0.13±1.4**</td>
<td>0.72±1.5*</td>
</tr>
</tbody>
</table>

Each value is the mean ± standard error of the mean.

*** Highly significant at P≤0.001, ** Significant at P≤0.01, *Low significant at P≤0.05.


