EVALUATION OF THE BIOACTIVITY OF CYANOBACTERIAL EXTRACTS ON ALBINO RATS
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4.1: INTRODUCTION

The intracellular biochemical constituents of the marine cyanobacteria, are rich and varied. It will not be possible to go into the bioactivity of each one of them, in view of the limitations imposed by time factor and facilities available. Hence, in the present study, only the water soluble fraction of the alcoholic extracts of the various strains was assessed; it is known that most of the biologically active principles are obtained in this fraction. Therefore, the study is not exhaustive, but only can provide a lead for future investigations.

The animal system chosen was a Wistar strain male albino rats, established as the most convenient mammalian system, closer to the human system for biological activity studies. In the present study, only male rats were used so as not to enter into the complex situation prevailing in the female rats connected with the cyclicity of the reproductive mechanism.

Drugs/toxins/nutritional supplements may affect any system in the body or any of the physiological mechanisms. However, invariably any such change is reflected in the blood, therefore, in the present investigation, analysis is restricted to the blood (Inwood and Thomson, 1975). Even here, the analysis is not exhaustive, but only representative in that haematological parameters, major biochemical constituents and those ingredients which are under the homeostatic control mechanisms have been investigated. However, attempt was also made to test the toxicity by analysing the levels of specific
aminotransferases viz. serum glutamic oxalacetic transaminase and serum glutamic pyruvic transaminase.

4.2: MATERIALS AND METHODS

4.2.1: TEST ANIMAL AND ITS MAINTENANCE IN THE LABORATORY

To evaluate the cyanobacterial bioactivity on the animal system, male albino rat of Wistar strain (Rattus norvigicus), weighing 100 to 150 g, obtained from Fredrick Institute of Plant Protection and Toxicology, Padappai, Madras, (FIPPT) was used as the test animal. Rats were kept in cages (18"x12"x6") provided with wire mesh on the top and housed in a well ventilated, temperature controlled (29±2°C) room. Each cage was provided with husk bed which was periodically changed to maintain the hygiene. The rats were fed with standard balanced pellet diet (Lipton India Ltd.) and drinking water was made available ad libitum.

4.2.2: CYANOBACTERIAL EXTRACT PREPARATION

Thirty days old cyanobacterial biomass, cultured as mentioned in the chapter II, were filtered, washed thoroughly in distilled water and homogenized separately in 95% ethanol. The extracts were separated by centrifugation at 2000 x g for 15 min. Extraction procedure was repeated for three times until the colour of the biomass became pale, implying the complete removal of the entire ethanol soluble compounds. The collected supernatants of each strain were pooled and dried by flushing pure nitrogen gas. The dry powder was weighed and
Plate - 3

Wistar strain of male albino rat
reconstituted in phosphate buffered saline (PBS, NaCl 0.8%; K$_2$HPO$_4$ 0.12% and KH$_2$PO$_4$ 0.034%; pH 7.3) where the concentration was adjusted to 10 mg dry weight/mL and the solution was preserved in a deep freezer.

4.2.3: TREATMENT

After laboratory acclimatization for seven days, the rats were divided into 13 groups, each consisting of 5 rats of identical size and known weight and treated as follows. The group-1 served as the control and the rats were administered intra-peritonally (i.p.), 0.5 mL of PBS in the thigh, daily for 7 days. Rats of the experimental groups were administered 0.5 mL of the extracts daily for 7 days, each group receiving the extract of one particular strain of the cyanobacteria. The administration was done every day between 11.00 am and 12.00 noon. The rats were harvested on the 8th day at the same time interval.

4.2.4: FOOD INTAKE AND BODY WEIGHT CHANGES

After the administration, the rats were housed in separate cages and had access to feed on a known quantity of the pellet feed. From the 2nd day onwards, the left over of the feed was weighed, so also the rats. The values thus obtained were used to deduce the daily feed intake and change in the body weight if any, of the rats.
4.2.5: BLOOD COLLECTION AND SERUM SEPARATION

After cutting the tip of the tail using a sharp sterilized scalpel, the tail was milked to allow blood to ooze out. The first drop of the blood was wiped off using cotton moistened with 70% alcohol. Subsequent drops were collected on clean slides and prepared into smears.

The rats were then anesthetized mildly using diethyl ether (vapour inhalation, using an ether cone) and dissected to expose the jugular vein. The vein was then punctured and the blood was collected in three sets of tubes. In the first instance, the blood was collected into small test tubes (6.0 x 0.5 cm) upto 0.5 mL mark and blood was used to record the clotting time. Subsequently, the blood was collected in a small vial to which a few crystals of EDTA (Ethylenediaminetetraacetic acid) were added. The blood was used for counting the blood cells and for the estimation of haemoglobin. The remaining blood was collected in a centrifuge tube and allowed to clot at room temperature. After clotting, the blood was centrifuged at 2000 x g for 15 min and the serum was separated. The serum was frozen for use in biochemical analysis.

4.2.6: RED BLOOD CELL COUNT (RBC) (Raphael, 1976)

To count red blood cell, the diluting fluid was prepared by mixing 10 mL of 40% (w/v) formaldehyde and 990 mL 3% (w/v) trisodium citrate.
Neubauer counting chamber and cover glass were cleaned and coverglass was kept in position over the ruled area using gentle pressure. EDTA treated blood was drawn exactly to the 0.5 mark in RBC pipette followed by the diluting fluid to the 101 mark and the pipette was rotated thoroughly. The counting chamber was filled with diluted blood and cells were allowed to settle for 3 min. Number of red blood cells were counted under microscope in 80 small squares (four squares of 16 each at the four corners and one of 16 at centre). The same procedure was repeated for 5 times per sample and average was taken for calculation. Total number of red blood cells was computed using the following formula: $X \times 10^4$ per ul of blood, where $X$ was total number of cells counted in 80 small squares.

4.2.7: WHITE BLOOD CELL - TOTAL COUNT (WBC-TC) (Raphael, 1976)

The white blood cell diluting fluid was prepared by mixing 3 mL of glacial acetic acid with 97 mL of distilled water and gentian violet was also added to give a pale violet colour.

The EDTA treated blood was drawn upto the 0.5 mark followed by the diluting fluid to the mark 11, and the pipette was shaken thoroughly. The Neubauer chamber was filled as mentioned earlier. Leukocytes were counted in the four corner blocks of Neubauer counting chamber under microscope. The total number of WBC was computed using the formula: $X \times 50$ per ul of blood, where $X$ was total number of cells in the four corner squares.
4.2.8: WHITE BLOOD CELL - DIFFERENTIAL COUNT (WBC-DT)
(Raphael, 1976)

The smear of the blood was prepared as previously described. Leishman's stain was used to differentiate the leukocytes. The stain was prepared by adding 0.15 g of Leishman powder in 100 mL of methanol and preserved in the refrigerator.

The dried blood film was kept on the staining rack and was flooded with Leishman's stain. After 3 min, distilled water was poured and allowed to stain for 15 min and the excess stain was removed by washing with the distilled water for 2 min. The smear was then dried in air and examined under the oil. Different white blood cells were identified based on the size, shape of the nucleus and colour of the cytoplasm. Totally, hundred cells were identified and the relative occurrence of each type was expressed in percentage.

4.2.9: BLOOD PLATELET COUNT (Raphael, 1976)

Platelet diluting fluid was prepared by mixing sodium citrate 3.8 g, formalin 0.2 mL and brilliant cresyl blue 0.1 g in 100 mL of distilled water. The methodology for platelet count was the same as adopted for RBC count. The results were expressed as total number of cells per uL of blood.
4.2.10: ESTIMATION OF BLOOD HAEMOGLOBIN (Oser, 1965)

Haemoglobin was estimated by cyanomethemoglobin method. The reagent was prepared by mixing potassium ferricyanide 200 mg and potassium cyanide 50 mg in 1 litre distilled water.

20 μL of EDTA treated blood was taken, to which 5 ml of the reagent was added and mixed thoroughly. This was kept at room temperature for 30 min and absorbancy was measured at 540 nm in Spectrophotometer (Milton and Roy, USA) against the reagent blank. The standard contained 60 mg haemoglobin. Haemoglobin content was calculated from the formula.

\[
\text{Abs. of unknown} \times \frac{\text{mg haemo}}{\text{Abs. of standard}} = \frac{\text{mg haemo}}{\text{standard}} \times \frac{\text{g/100 mL}}{0.251}
\]

4.2.11: Mean corpuscular haemoglobin (MCH) (Raphael, 1976)

To determine the average haemoglobin content in a single red blood cell was commuted from the following formula.

\[
\text{MCH in pg} = \frac{\text{Haemoglobin content}}{\text{RBCs in million per μL}} \times 10
\]
4.2.12: BLOOD CLOTTING TIME (Davidson and Nelson, 1974)

To find the clotting time, blood was collected separately as already described (duplicate samples for each rats). The time of reading was noted and the tubes were tilted periodically to observe clotting. The first instance when the tube could be tilted periodically to horizontal position when the blood did not exhibit tendency to flow out, was taken as the inference of clotting and the time there upon was noted. Average of the time for clotting of the two samples collected from the each rat was calculated.

4.2.13: SERUM PROTEIN (Lowry et al., 1951):

Reagents:

a) 2% sodium carbonate in 0.1 N sodium hydroxide.
b) 0.5% copper sulphate in 1% sodium potassium tartarate solution (prepared fresh).
c) Mixed 50 mL of reagent-a and 1 mL of reagent-b, just prior to use.
d) Folin-Ciocalteau reagent: commercially available reagent diluted with equal volume of water just before use.

10 uL of serum was taken and diluted to 1 mL in a clean test tube to which 5 mL of alkaline mixture (reagent-c) was added. After standing at room temperature for 10 min, 0.5 mL of reagent-d was added and mixed rapidly. Tubes were left as such for 30 min and the
absorbancy was measured at 650 nm in the Spectrophotometer against a reagent blank. A standard graph was prepared by using bovine serum albumin (BSA) at concentrations ranging from 30 to 300 ug/mL at 30 ug interval. The protein present in the serum was commuted from the standard graph and the result was expressed in ug/mL.

4.2.14: SERUM FREE SUGARS (Method of Folin & Wu, Oser, 1965)

Reagents:

a) Alkaline copper solution: 40 g of pure anhydrous sodium carbonate was dissolved in 400 mL of distilled water and 7.5 g of tartaric acid was added to it. There after, 4.5 g of crystalline copper sulphate was dissolved and made to one litre using distilled water.

b) Phosphomolybdic acid solution: To 200 mL of 10% sodium hydroxide solution, 35 g of molybdic acid, 5 g of sodium tungstate and 200 mL of distilled water were added, boiled vigorously for 20 to 40 min and then cooled immediately. The concentrate was diluted to 350 mL with distilled water and 125 mL conc. phosphoric acid (85%) was added. The resultant solution was made upto 500 mL with distilled water.

c) Tungstic acid solution (10%): 100 g of carbonate free sodium tungstate was dissolved in one litre of distilled water.

d) 2/3 N sulphuric acid: 35 g of conc. H₂SO₄ was diluted to 1000 mL with distilled water.
Two mL of serum and 16 mL of water were mixed thoroughly. Subsequently, one mL each of reagent-c and d were added. The preparation was allowed to stand for 10 min and the protein free supernatant was saved after centrifugation at 1000 x g for 10 min.

In a clean Folin-Wu sugar tube, 2 mL of tungstic acid serum was taken and 2 mL of reagent-a was added. The tube was kept in a boiling water bath exactly for 8 min. The tube was then cooled in running water bath and 2 mL of reagent-b was added. After one min, the content was diluted up to the 50 mL mark of the tube and mixed thoroughly. The reagent blank was also processed simultaneously. The absorbancy was measured at 420 nm in the Spectrophotometer. The standard graph was plotted by using D-glucose ranging in concentration from 100 ug to 1.0 mg/mL at 100 ug interval. The free sugar level of serum sample was deduced from the standard graph and value was expressed as mg/100 mL of serum.

4.2.15: SERUM CHOLESTEROL (Wybenga 1970).

A clinical kit was procured from Siddham diagnostics, Nagpur, India. The kit consisted of two reagents R1 & R2, where R1 was cholesterol reagent and R2 was cholesterol standard (200 mg%).

5 mL of cholesterol reagent (R1) was taken in a clean tube and 0.05 mL of serum was added. The tube was placed in a boiling water bath for exactly 90 seconds and then cooled immediately in
running tap water for 5 min. The absorbancy was measured at 560 nm in the Spectrophotometer against a reagent blank. The standard contained 200 mg cholesterol per 100 mL. The cholesterol content of the serum was calculated using the formula.

\[
\text{Abs. of unknown} - \text{Abs blank} = \frac{\text{Abs. of standard} - \text{Abs blank}}{200} \times \text{Cholesterol (mg/100mL)}
\]

4.2.16: SERUM CALCIUM AND MAGNESIUM (Mackareth, 1963)

Reagents:

a) EDTA titrant (0.1N): prepared in distilled water and stored in polyethylene bottle.

b) Ammonium buffer: 70 g of ammonium chloride dissolved in 570 mL of ammonia solution.

c) 80% sodium hydroxide solution in distilled water.

d) Eriochrome black T indicator (Solochrome black T): A fine mixture of eriochrome black T and sodium chloride, prepared in the ratio 1:200 w/w, by grinding in a mortar and pestle.

e) Ammonium purpurate (Muraxide) Indicator: A mixture was prepared by mixing muraxide and sodium chloride in the ratio 1:100 w/w.

f) 10% (w/v) Trichloroacetic acid (TCA) in distilled water.

To one mL of serum in a test tube, one mL of 10% TCA was added and shaken thoroughly. The white precipitate was sedimented by
centrifugation at 2000 \times g \text{ for 15 min.} \text{ The deproteinized supernatant was used for further analysis.}

1 \text{ mL of deproteinized serum was pipetted onto 50 mL conical flask and 5 mL of ammonium buffer was added and diluted to 20 mL with distilled water. A pinch of eriochrome black T was added and the solution was warmed to 60^\circ C. This was titrated against EDTA until the red colour turned to permanent blue and the end point was noted as 'A'.}

In another flask one mL of deproteinized serum and one mL of NaOH solution were taken and diluted to 20 mL with distilled water. A pinch of muraxide indicator was added and titrated against EDTA until the pink colour turned to permanent blue. The end point was noted as 'B'.

\[
\text{Consumption of EDTA by Ca and Mg} = A \\
\text{Consumption of EDTA by Ca alone} = B \\
\text{Consumption of EDTA by Mg alone} = A - B
\]

\[
F \times B \times 100
\]

\text{Amount of calcium in sample, mg/100 sample volume}

\[
F \times (A - B) \times 100
\]

\text{Amount of Magnesium in sample, mg/100 mL sample volume}
4.2.17: SERUM SODIUM AND POTASSIUM (Wetzel and Likents, 1979)

Sodium and potassium were determined using a flame photometer (courtesy: Regional Rice Research Institute, Aduthurai, Tamilnadu). Sodium standard graph was prepared from 100 to 160 milliequivalents of sodium per litre, at 10 milliequivalent interval, using sodium chloride. Potassium standard graph was prepared using 3 to 7 milliequivalents of potassium per litre at 1 milliequivalent interval, using potassium chloride. The serum sample was deproteinized using TCA and aspirated in flame photometer and the readings were noted. From the standard graphs values of the test samples were calculated and expressed in ppm.

4.2.18: SERUM GLUTAMATE OXALOACETATE TRANSAMINASE (SGOT): (Reitman & Frankel, 1957)

L - Aspartate: 2-oxoglutarate aminotransferase.

E.C.2.6.1.1

\[ \text{GOT} \]

\[ \alpha\text{-Ketoglutarate} + \text{L-Aspartate} \quad \longrightarrow \quad \text{L-Glutamate} + \text{Oxalacetate} \]

Reagents:

a) Buffered aspartate (pH 7.4) prepared by the addition of 0.292 g of \( \alpha \)-ketoglutaric acid and 26.6 g DL-aspartic acid 0.1 N NaOH was slowly added until all solids dissolved.
b) 2,4-Dinitrophenyl hydrazine solution: 0.198 g of 2,4-dinitrophenyl hydrazine was dissolved in 1 N HCl and made up to 1000 mL.
c) 0.4N NaOH.
d) 2 mM Pyruvic acid standard.

(A diagnostic kit was purchased from, the Span Diagnostics private limited, India)

0.5 mL of reagent was pipetted into a clean tube and was incubated at 37°C for 5 min. Immediately, 0.1 mL of serum was added and incubated at 37°C for 60 min. 0.5 mL of reagent-b (colour reagent) was added and allowed to stand at room temperature for 20 min. Thereafter, 5 mL of reagent-c was added and the absorbancy was measured at 505 nm in the Spectrophotometer against distilled water as blank.

The standard graph was prepared adopting the procedure as above, but using 0.5, 0.45, 0.4, 0.35 and 0.3 mL of buffered aspartate (reagent-a) and 0.0, 0.05, 0.1, 0.15 and 0.2 mL of the working pyruvate standard (2mM per litre) corresponding to 0, 24, 61, 114 and 190 units of enzyme activity per mL from the reagent-a and using distilled water in the place of serum. The units of enzyme activity was obtained using the standard graph.

4.2.19: SERUM GLUTAMIC PYRUVIC TRANSAMINASE (SGPT): (King and Jagatheesan 1959)

L-Alanine: 2-oxoglutarate aminotransferase

E.C. 2.6.1.2.
GPT
\[
\alpha - \text{Ketoglutaric acid} + \text{Alanine} \rightarrow \text{Pyruvic acid} + \text{Glutamic acid}
\]

Reagents:

a) Buffered substrate: Available in the kit
b) 2, 4-Dinitrophenyl hydrazine solution: As in 4.2.18 above
c) 0.4 M sodium hydroxide solution
d) 2mM sodium pyruvate solution

The procedure was the same as mentioned in 4.2.18 above, with the incubation time fixed at 30 min. The results were expressed in enzyme activity units/mL.

(A diagnostic kit was purchased from the Ortho Diagnostics private limited, India)

4.2.20: SERUM ACID PHOSPHATASE (ACP) (Varley, 1980)

Orthophosphoric-monoester phosphohydrolase

(E.C. 3.1.3.2)

ACP

\[
\text{Phenyl phosphate} \rightarrow \text{Phenol} + \text{Inorganic phosphate}
\]
Phenol + 4-aminooantipyrine → Orange-red coloured complex ($\lambda_{\text{max}}=510\,\text{nm}$)

Reagents:

- **a)** Buffered substrate pH 4.9: A standard tablet was dissolved in 4 mL of distilled water and mixed well.
- **b)** 0.5N sodium hydroxide
- **c)** 0.5N sodium bicarbonate
- **d)** 0.6% 4-aminooantipyrine
- **e)** 2.4% potassium ferricyanide
- **f)** 1 mg% phenol standard

(A diagnostic kit was purchased from the Span diagnostics private limited, India)

One mL of reagent-a was pipetted on to a clean test tube and 1 mL of distilled water was added. The solution was mixed well and incubated at 37$^\circ$C for 3 min. Immediately after incubation, 0.2 mL of serum was added and incubated at 37$^\circ$C for 60 min. Thereafter, 1.0 mL each of reagents b, c, d and e were added one after another accompanied by thorough mixing. The final colour was read at 510 nm in the Spectrophotometer.

Simultaneously, blank, standard and control were processed by following the same procedure with a few modifications. In the blank, reagent-a was replaced by distilled water and in the standard, instead of reagent-a, phenol standard was added. For control, the procedure was
the same as test sample, but serum was added only after the addition of reagent-b. The enzyme activity was calculated using the following formula and the values were expressed in KA units.

\[
\text{ACP (KA units)} = \frac{\text{Abs. test} - \text{Abs. control}}{\text{Abs.Standard} - \text{Abs.blank}} \times 5
\]

4.2.21: SERUM ALKALINE PHOSPHATASE (ALP): (Kind and King, 1954)

Orthophosphoric-monoester phosphohydrolase (alkaline optimum) (E.C.3.1.3.1)

\[
\text{ALP} \quad \text{Phenyl phosphate} \rightarrow \text{Phenol + Inorganic phosphate}
\]

\[
\text{phenol + 4-aminoantipyrine} \rightarrow \text{orange-red coloured complex (} \lambda_{\text{max}}=510 \text{ nm)}
\]

Reagents:

a) Buffered substrate pH 10.0: A standard tablet was dissolved in 3.0 mL of distilled water.

b) 0.5N sodium hydroxide.

c) 0.5N sodium bicarbonate.

d) 0.6% 4-aminoantipyrine.

e) 2.4% potassium ferricyanide.

f) 1 mg% phenol standard.
(A diagnostic kit was purchased from the Span Diagnostics private limited, India)

one mL of reagent-a was taken in a clean test tube and diluted to one mL with distilled water. The mixture was incubated at $37^\circ\text{C}$ for 3 min. Immediately after incubation, 0.1 mL of serum was added directly to the substrate and incubated at $37^\circ\text{C}$ for 15 min. After incubation, 0.8 mL reagent-b, 1.2 mL of reagent-c, 1 mL of reagent-d, and 1 mL of reagent-e were added by thorough mixing.

The blank, standard and control were also processed simultaneously. The enzyme activity was calculated using the following formula and the values were expressed in KA units.

\[
\text{ALP (KA unit)} = \frac{\text{Abs.test} - \text{Abs. control}}{\text{Abs. standard} - \text{Abs. blank}} \times 10
\]

4.2.22: Statistical analysis (Zar, 1984)

The experimental data were analysed statistically to get a clear idea about the trend of the results. Different statistical tools like mean, standard deviation, standard error and Student's 't' test were used in the present study.
A. The Arithmetic Mean: It is simply referred as the mean, and calculated as the sum of all the Xi values divided by the size of the population (n).

\[ \bar{X} = \frac{\sum X_i}{n} \]

where \( \bar{X} \) = mean
\( \sum \) = summation

B. Standard deviation: Standard deviation is the positive square root of the variance and it was calculated from the following formula.

\[ \sigma = \sqrt{\frac{\sum (X - \bar{X})^2}{n-1}} \]

\( \sigma \) = Standard deviation
\( \sum \) = Summation
\( X, \bar{X} \) = Mean values of variance
\( n \) = Size of the population

C. Student 't' test:

To calculate 't' value, following formula was employed.
difference of mean values
\[ t = \frac{\text{difference}}{\sqrt{\text{SE}_1^2 + \text{SE}_2^2}} \]

SE = Standard error \((\text{SD}/\sqrt{n})\)

The calculated \(t\) value was compared with the critical values of the \(t\) distribution table with the corresponding degrees of freedom. In all the statistical comparison of data \(p\) value less than 0.05 was considered significant, meaning the variation was really due to treatment and not because of experimental error.

4.3: RESULTS AND DISCUSSION

4.3.1: BODY WEIGHT AND FOOD INTAKE

The weight of the control rats increased gradually from 149 g before commencing the experiment to 174 g on the day of harvest (Fig 3c). Though, between any two consequent days or even between the terminal days, the difference was not statistically significant. This increase in the body weight can be attributed to growth, in view of the age of the rats used in the investigation (two months old). With *Sy. elongatus* BDU 30312, *S. subsalsa* BDU 30311, *O. salina* BDU 10142, *P. valderianum* BDU 20571, *P. angustissimum* BDU 40061, *P. corium* BDU
FIG-3: GRAPHS SHOWING THE CHANGE IN WEIGHT OF THE RATS ON TREATMENT WITH THE EXTRACTS OF THE VARIOUS CYANOBACTERIAL STRAINS CORRESPONDING TO THE FEED INTAKE

...{(contd.)}
FIG-3:

*Synechococcus elongatus BDU 30312*

- Animal weight in grams
- Feed intake in grams

*Spirulina subsalsa BDU 30311*

- Animal weight in grams
- Feed intake in grams

*Oscillatoria salina BDU 10142*

- Animal weight in grams
- Feed intake in grams

*Oscillatoria formosa BDU 40261*

- Animal weight in grams
- Feed intake in grams
FIG-3:

Phormidium valderianum BDU 20571

Phormidium angustissimum BDU 40061

Phormidium corium BDU 30241

Phormidium tenue BDU 41001

...(contd.)
FIG-3:

Lyngbya sp. BDU 30601

Plectonema terebrans BDU 30342

Pseudanabaena schmidlei BDU 30313

Dichothrix baueriana BDU 40481
30241, P. tenue BDU 41001, Pl. terebrans BDU 30342 and D. baueriana BDU 40481, there was an increasing trend in body weight, though with minor fluctuations, whereas with O. formosa BDU 40261, Lyngbya sp. BDU 30601 and Ps. schmidlei BDU 30313 (Figs-3 and 4) the trend showed decrease in body weight with advancing days.

The feed intake of the control rats ranged from 11.25 to 15.5 g per day per animal (Fig 3 and 4) with a fluctuating trend. The treatment did not appear to affect the feed intake with S. subsalsa BDU 30311, O. salina BDU 10142, P. valderianum BDU 20571, P. angustissium BDU 40061, P. tenue BDU 41001, Pl. terebrans BDU 30342 and D. baueriana BDU 40481. On the contrary, with Sy. elongatus BDU 30312 and O. formosa BDU 40261 the feed intake increased, whereas, with P. corium BDU 30241, Lyngbya BDU 30601 sp and Ps. schmidlei BDU 30313, it decreased. The increase in the feed intake was more pronounced with Sy. elongatus BDU 30312, whereas, the decrease was more pronounced with Lyngbya sp BDU 30601, and Ps. schmidlei BDU 30313 (Figs-3 and 4).

Comparing the change in the body weight and feed intake with O. formosa BDU 40261, the body weight decreased though the feed intake increased slightly, suggesting that this strain enhanced the consumption, but affected conversion. On the other hand, Lyngbya sp BDU 30601, and Ps. schmidlei BDU 30313 appear to deter food intake and thus contribute negatively to growth. An interesting observation made with S. subsalsa BDU 30311 is that, whereas the feed intake maintained almost a steady state, the body weight increased as in the
Number indications used in figures

C = Control
1 = Sy. elongatus BDU 30312
2 = S. subsalsa BDU 30311
3 = O. salina BDU 10142
4 = O. formosa BDU 40261
5 = P. valderianum BDU 20571
6 = P. angustissimum BDU 40061
7 = P. corium BDU 30241
8 = P. tenue BDU 41001
9 = Lyngbya sp. BDU 30601
10 = Pl. terebrans BDU 30342
11 = Ps. schmidlei BDU 30313
12 = D. baueriana BDU 40481
FIG-4:

BAR DIAGRAMS SHOWING THE TRENDS IN FEED INTAKE AND BODY WEIGHT CHANGE OF RATS ON TREATMENTS WITH VARIOUS CYANOBACTERIAL EXTRACTS
control, suggesting that this strain of cyanobacterium contributed to conversion efficiency without affecting feed intake. This could be attributed to the rich protein content of this strain which would have supplemented the diet, because in all the other cases of increase in body weight, it coincided with increased consumption of food.

4.3.2: RED BLOOD CELL COUNT (RBC COUNT)

The control rats recorded $6.5 \pm 0.20$ RBC per $\mu$L of blood. Treatment of rats with the strains *S. subsalsa* BDU 30311, *O. formosa* BDU 40261, *P. valderianum* and *P. angustissimum* BDU 40061 did not affect the RBC count. However, treatment with *Sy. elongatus* BDU 30312, *O. salina* BDU 10142, *P. tenue* BDU 41001, *Lyngbya* sp BDU 30601, *Ps. schmidlei* BDU 30313 and *D. baueriana* BDU 40481 resulted in increased RBC count; as calculated from the mean values, the percentage difference from the control was 23, 31, 23, 31, 15 and 29% respectively (calculated for the mean) and the differences were statistically significant. On the other hand, the treatment with *P. corium* BDU 30241 and *Pl. terebrans* BDU 30342 resulted in decrease in the RBC count to the extent of 20% and 17% respectively from the control value (calculated from the mean) (Fig-5A).

RBCs are a part of the homeostatic system and their number in circulation is maintained constant under normal circumstances (Thompson, 1975). Any change in the RBC count, particularly on the negative side, reflects a pathological state (Inwood and Thomson, 1976). From this viewpoint, it can be said that strain *P. corium* BDU 30241 and
BAR DIAGRAMS REFLECTING THE CHANGES IN THE VARIOUS BLOOD PARAMETERS STUDIED TO ASSESS THE BIOACTIVITY OF THE VARIOUS CYANOBACTERIAL EXTRACTS

( $p < 0.05$, wherever applicable )

A. RED BLOOD CELL COUNT

B. BLOOD HAEMOGLOBIN CONTENT

C. MEAN CORPUSCULAR HAEMOGLOBIN
*Pl. terebrans* BDU 30342 contain a factor which affects erythropoiesis and may be probably toxic. Any treatment which leads to decreased RBC count would cause problem in transporting the respiratory gas appropriately, leading to a state of anaemia (Nath, 1989). However, the constituent of the cyanobacterial strains *P. coriun* BDU 30241 and *Pl. terebrans* BDU 30342 which are responsible for the decrease in the RBC count if identified, can be applied in the treatment of patients whose RBC count is above normal under pathological conditions.

On the same logic, it can be said that strains *Sy. elogatus* BDU 30312, *O. salina* BDU 10142, *P. tenue* BDU 41001, *Lyngbya* sp. BDU 30601, *Ps. schmidlei* BDU 30313 and *D. baueriana* BDU 40481, each contained a factor(s) which contributed to increase in RBC count. Though, at the outset, increase in RBC count would apparently mean positive, actually such an increase is also not desirable, in view of the consequent increase in the density of the blood and haemococoncentration. In general, any abnormal increase in RBC count is also a pathological state and it is known as polycythemia (Inwood and Thomson, 1975). However, the causative factors in these cyanobacterial strains, if identified, will help in their use as a drug against those pathological situations which tend to decrease the RBC count (haemopoietic anaemia).
4.3.3: BLOOD HAEMOGLOBIN CONTENT AND MEAN CORPUSCULAR HAEMOGLOBIN (MCH)

The haemoglobin content of the blood of control rats were recorded at 15.57 ± 0.15 (Fig-5B). Treatment with the various cyanobacterial extracts resulted in significant decrease in blood haemoglobin content, the percentage of difference ranging from 4.11 to 28.26. The MCH of haemoglobin of the control rat was 24 pg. The treatments invariably resulted in decrease in the MCH, the difference ranging from 6.26 to 41.04%, excepting in the case of *Pl. terebrans* BDU 30342, where it increased to 6.6% above the control value (Fig-5C).

The normal range of blood haemoglobin of man is 14.5 to 17 g/100 mL (Oser, 1965) and rats maintain a slightly lower value (Johri, 1990). The MCH in man is 27 to 32 pg (Inwood and Thomson, 1975). Any marginal decrease in the blood haemoglobin content as seen in the present study, cannot be considered to be at anaemic level (Johri, 1990). The changes in the MCH also do not reflect any toxic effect of the cyanobacteria. Thus, with regard to blood haemoglobin content and MCH the 12 strains studied were at the safe level (Fig-5C).

4.3.4: WHITE BLOOD CELL - (TOTAL and DIFFERENTIAL COUNT)

WBC count of control rats was 5.96 ± 0.07 per μL of blood. Treatment of rats with the various cyanobacterial extracts altered the total WBC count: whereas, treatment with the strains *Sy. elongatus* BDU 30312, *S. subsalsa* BDU 30311, *O. formosa* BDU 40261, *P.
angustissimum BDU 40061, P. tenue BDU 41001, Pl. terebrans BDU 30342, Ps. schmidlei BDU 30313 and D. baueriana BDU 40481 resulted in an increase in the WBC count, with O. salina BDU 10142, P. valderianum BDU 20571 and P. corium it was decreased (Fig 5D).

Lymphocyte, neutrophil and eosinophil were the leukocyte types distinguishable in the blood of rat; monocyte and basophil were rare and negligible. The blood of control rat contained 83.7% of lymphocytes, 14.1% of neutrophils and 1.8% of eosinophils (all mean values). Lymphocytes are the predominant leukocyte type which decreased in all the cases of cyanobacterial treatments, but significantly only with the strains Sy. elongatus BDU 30312, S. subsalsa BDU 30311, O. formosa BDU 40261, P. angustissimum BDU 40061, P. corium BDU 30241, P. tenue Lyngbya sp. BDU 30601, Pl. terebrans BDU 30342 and Ps. schmidlei BDU 30313 (Fig-5 E). On the other hand, neutrophil count was increased significantly almost in all the cases excepting in the treatment with the strain P. valderianum BDU 20571, where it decreased significantly (Fig-5 E). The eosinophil count increased significantly on treatment with Sy. elongatus BDU 30312, S. subsalsa BDU 30311, P. angustissimum BDU 40061, P. corium BDU 30241 and P. tenue BDU 41001 and decreased significantly on treatment with P. valderianum BDU 20571 only; in all other cases the deviation from the mean was not significant (Fig-5 E).

Changes in the leukocyte number reflect the leukocytes' response to systemic conditions. Lymphocytes are the predominant leukocyte cell type and are agranulocytes. They are concerned with the
FIG-5

WHITE BLOOD CELL TOTAL COUNT

NO. OF CELLS X 1000 PER UL OF BLOOD

CYANOBACTERIAL SPECIES

D

WHITE BLOOD CELL DIFFERENTIAL COUNT

LYMPHOCYTE

NEUTROPHIL

EOSINOPHIL

CYANOBACTERIAL SPECIES

E
immune response (Thompson, 1975). There are two main types of lymphocytes viz., T (thymus) and B (bone marrow) cells. T-cells are mainly related to the cellular immunity and B cells to humoral immunity (Weir, 1983). The observation in the present study, reflected decreased immune response. It is generally known that the lymphocytes decrease in count in response to several conditions like stress, administration of corticosteroid, thyroid diseases, chemotherapeutic agents, etc. (Talwar, 1989). In the present situation, the decrease in the count of lymphocytes can be attributed principally to stress or augmentation of the output of corticosteroid either directly or through the stimulation of pituitary ACTH; the stress induced by the administration of a foreign substance in itself causative of stimulation of ACTH - corticosteroid system. Rats with decreased lymphocyte count due to the experimental condition, though would apparently reflect suppressed immune response, may not be reflecting the direct effect of any of the ingredients in the extract, but probably the stress factor. It is said that lymphocyte count of less than 500/uL is only alarming and will lead to the pathological state known as 'lymphopenia' (Inwood and Thomson, 1976). In none of the groups of rats treated with the extract of the various cyanobacteria, the number decreased to the comparable alarming state, again leading to the inference that their ingredients did not cause lymphopenia.

On the contrary, the treatments resulted in increase in the neutrophil count, excepting in the case of P. valderinum BDU 20571 (Fig-5E). Neutrophils are immobile while in blood and with appropriate stress, they mobilize in large number from the bone marrow and converge on the affected area for the phagocytic action (Inwood and
Thomson, 1976). They are the most important cell in cellular defence in the body (Roitt, 1988). Therefore, the results in general, reflect the stimulation of cell-mediated immune response which in itself is an indication of stress factor. However, in no case was the count is excess of 6000/μL which is normally the pathological state known as 'neutrophilia' (Inwood and Thomson, 1976). The change in the eosinophil count is also not alarming, because only a count more than 400/μL is indicative of a pathological state and in the present work, in no instance was this number obtained.

Thus, the response of the different leukocytes to the treatments emphasise more the stress factor than any positive or negative direct response to the ingredients of the cyanobacterial extracts. If this assumption is pertinent and proved in the affirmative through specific investigations designed in future for this purpose, it may be concluded that, these cyanobacteria can be used without any hesitation in the animal system, probably including man, for the desired results in any other physiological mechanism.

4.3.5: PLATELET COUNT AND BLOOD CLOTTING TIME

The results in general reflect a direct relationship between the platelet count and blood clotting time. The blood of control rats contained 1.34 x 10^6 platelets per μL of blood and the blood clotting time was 17.6 sec (both mean values). The platelet count decreased with the strains Sy. elongatus BDU 30312, S. subsalsa BDU 30311, O. salina BDU 10142, P. tenue BDU 41001, Pl. terebrans BDU 30342 and Ps.
*schmidlet* BDU 30313 and it is interesting that in all these cases the blood clotting time increased. On the other hand, with the strains *O. formosa* BDU 40261 and *Lyngbya* sp.BDU 30601, the platelet count increased and the clotting time decreased (Figs-1-5 F and G). However, with almost all the strains the deviation in the platelet count as well as blood clotting time was only marginal, while with *S. subsalsal* BDU 30311 the situation was alarming in that, the platelet count decreased to less than 50% of the control value and the clotting time increased more than 4 times. Thus, of the strains affecting platelet count and blood clotting *S. subsalsal* BDU 30311 needs to be viewed with interest.

In view of the fact that the alga *Sargassum linifolium* has been reported to contain high anticoagulant activity [the substance identified as sargassan, a sulphatized polysaccharide (SPS)], (Abdel-Fattah et al., 1974) and *Ecklonia, Laminaria, Hizikia, Lassonia, Myagropsis* and other *Sargassum* species have been reported to contain fucose containing SPS influencing various blood coagulating test models (Nishino and Nagumo, 1987), to test the possibility of a substance in *S. subsalsal* BDU 30311 which would directly affect blood clotting, the following test was conducted.

Blood of untreated rats, was collected in a vial containing a drop of a *S. subsalsal* BDU 30311 extract and the clotting was tested. There was no delay in the clotting. Thus, this test confirmed the absence of any direct anticoagulant in *S. subsalsal* BDU 30311.
FIG-5

BLOOD CLOTTING TIME

PLATELET COUNT

BLOOD CLOTTING TIME IN SECONDS

NO. OF CELLS (x 1000000) PER UL OF BLOOD

CYANOBACTERIAL SPECIES

CYANOBACTERIAL SPECIES
Haemostasis is a highly complex phenomenon occurring at the site of a wound to prevent the loss of blood or to stop bleeding. The various factors involved in haemostasis reaction and the sequence of reactions leading to the formation of the fibrin clot are shown in the figure (Inwood and Thomson, 1976). Essentially, the plasma factor called prothrombin is converted into active thrombin which in-turn reacts with the plasma protein fibrinogen to convert it into insoluble fibrin threads, which form at the site of the wound and into which first the platelets and then the red cells are entangled, forming the final clot (Gy Blasko and Puri, 1989).
From the foregoing discussion it is evident that platelets and blood clotting time are directly related. The results in the present study relating to the effect on clotting time and platelet count are clearly a reflection of this relationship. According to Inwood and Thomson (1976) any decrease in platelet count is due to decrease in megakaryopoiesis and the conditions that are implicated in this end result are congenital, drug-induced, malignant disease and megaloplastic anaemia. The situation obtaining with S. subsalsa BDU 30311, which deserves special treatment, can be explained as comparable to drug induced thrombocytopenia or to drug-induced bone marrow depression. This is explained in the immune complex disease theory, according to which, the drug becomes attached to the circulating serum antibody which in-turn becomes fixed to the surface of the platelet, causing its elimination (Inwood and Thomson, 1976). Therefore, it could be suggested that the extract of S. subsalsa BDU 30311 contains a principle or an ingredient which functions like a drug, affecting the platelet count and therefore, the clotting time. In this connection, it is necessary to point out that Spirulina in general is advocated as a nutritional supplement in view of its rich protein content (Seshadri and Thomas, 1979; Venkataraman and Becker, 1985). Therefore, further well designed investigations to find the particular principle responsible for affecting the platelet count and the clotting time, are highly pertinent.

4.3.6: SERUM PROTEIN

The control rats recorded 76.36 ± 3.92 µg of protein per mL of serum. Treatment with none of the extracts of 12 cyanobacterial
extracts resulted in any significant increase in the serum protein level. However, treatment with strains *S. subsalsa* BDU 30311, *O. formosa* BDU 40261, *P. corium* BDU 30241, *Lyngbya* sp. BDU 30601, *Pl. terebrans* BDU 30342 and *Ps. schmidlei* BDU 30313 resulted in a slight lowering of the serum protein level. But in no instance did the serum protein level decrease to less than 50 mg/mL which means, in no instance the decrease was to any alarming level (Fig-5 H).

Though the serum proteins are very important for maintaining the osmotic properties of the blood and are concerned with the immune response of the body, in the experimental treatment there was no ill effect. Therefore, cyanobacteria used in the present study can be applied to the animal systems as no dramatic change in serum protein level is contemplated.

### 4.3.7: BLOOD SUGAR

The sugar level of the serum of control rats recorded 114.20 ± 7.05 mg/100 mL. Administration of the extracts of *S. subsalsa* BDU 30311, *O. formosa* BDU 40261, *P. angustissimum* BDU 40061, *P. tenue* BDU 41001, *Pl. terebrans* BDU 30342 and *D. baueriana* BDU 40481 did not affect the blood sugar level. *Sy. elongatus* BDU 30312, *O. salina* BDU 10142, *P. valderianum* BDU 20571 and *P. corium* BDU 30241 caused a significant elevation of sugar level with the highest value obtaining with *P. valderianum* BDU 20571. Significant fall in the sugar level was recorded with *Lyngbya* sp BDU 30601. and *Ps. schmidlei* BDU 30313 (Fig-51).
Particularly, in-terms of the application of the cyanobacteria in the animal systems and in the human system, strains *Sy. elongatus* BDU 30312, *P. cortum* BDU 30241, *Lyngbya* sp BDU 30601, and *Ps. schmidlei* BDU 30313 appear to be important. The normal blood sugar level is reported as 80 to 120 mg/100 mL (Davidsohn and Nelson, 1977). Blood sugar level is under homeostatic regulation (Turner and Bagnara, 1976). Insulin produced by the islets of Langerhans acts to decrease the blood sugar level when there is hyperglycemia (sugar level above normal). At the hypoglycemic state, a series of hormones viz., adrenalin from the adrenal medulla, glucagon from the islets of Langerhans and glucocorticoids from adrenal cortex, are released in a sequence to bring the blood sugar level to normal. Under any state of experimental manipulation, when the blood sugar level is affected the hormones mentioned above are secreted appropriately so as to bring normoglycemia. Therefore, the results obtaining in the case of the six strains in affecting the blood sugar level, it is to be inferred that these cyanobacteria are capable of modifying the endocrine mechanisms of the animal.

Particularly, strains *Lyngbya* sp BDU 30601, and *Ps. schmidlei* BDU 30313 would be ascribed with clinical application, because a pathological state of the B-cells of the islets of Langerhans results in increase of blood sugar level above normal. Such patients, take periodical doses of external insulin to keep the sugar level under control (Lehninger, 1970). In decreasing the blood sugar levels, strains *Lyngbya* sp BDU 30601, and *Ps. schmidlei* BDU 30313 probably function like insulin or stimulate the B-cells of islets of Langerhans to increase
the output of insulin which could result in lowering of blood sugar level. Therefore, it is worth investigating further to identify the substance in the extracts of these two strains of cyanobacteria and to find the precise mechanism of action.

In increasing the blood sugar level, strains *Sy. elongatus* BDU 30312, *O. salina* BDU 10142, *P. valderianum* BDU 20571 and *P. corium* BDU 30241 may not have direct clinical application, because pathological states of hypoglycemia are rather rare (Henry, 1974). However, in terms of dietary application, their tendency to hyperglycemia even when combated by the native insulin, is interesting. Analysis of the total carbohydrate (Table-4) reveals that these four strains contain high level of intracellular carbohydrate, 23.70 g% in *Sy. elongatus* BDU 30312, 23.63 g% in *O. salina* BDU 10142, 22.26 g% in *P. valderianum* BDU 20571 and 19.44 g% in *P. corium* BDU 30241. The higher carbohydrate content of these four strains of the cyanobacteria, therefore correlates with the hyperglycemia in the rats administered with their extracts. Thus, these four strains of cyanobacteria appear to be of nutritional application.

4.3.8: SERUM CHOLESTEROL

Control rats recorded 108.87 ± 2.56 mg% cholesterol in the serum. Administration of the extracts of all the strains except *P. corium* BDU 30241 *D. baueriana*, resulted in a significant decrease in the serum cholesterol level, the percentage difference ranging from 8.05 to 33.94% below the control value. Particular mention needs to be made in this
context about the strains Sy. elongatus BDU 30312, S. subsalsa BDU 30311, O. salina BDU 10142, P. valderianum BDU 20571, P. tenue and Ps. schmidlet BDU 30313 in which a range of the blood cholesterol level was decreased by 22 to 34% of the control (Fig-5 J). Only one strain of cyanobacterium, P. cortum BDU 30241, tended to increase the blood cholesterol level significantly, but still not alarmingly (8.05% above the control value). *D. baueriana* BDU 40481 did not appear to affect the blood cholesterol level.

In increasing the cholesterol level to only 117.56 ± 6.68 mg%, though statistically significant, administration of *P. cortum* BDU 30241 still maintains the cholesterol well within the normal range. In decreasing the blood cholesterol level the seven strains of cyanobacteria used suggest clinical/dietary application. Cholesterol is a dynamic lipid in circulation and the blood cholesterol level is a reflection of the body lipid content. Hypercholesteronemia, is often a pathological state and would affect ultimately the heart function; cardiac failure is often related with hypercholesteronemia (Lehninger, 1970). While searching for nutritional supplement, it is always necessary that the dietary organism/substance does not increase the blood cholesterol level. In this context the cyanobacteria which in fact decrease the blood cholesterol level may fit in appropriately either as drugs or as favourable nutritional supplements.
4.3.9: SERUM CALCIUM

The control rats recorded 9.58 ± 0.34 mg calcium per 100 mL of serum. The calcium level decreased significantly in the rats administered with the extracts of the strains *Sy. elongatus* BDU 30312, *O. salina* BDU 10142, *P. angustissimum* BDU 40061, *Pl. terebrans* BDU 30342, *Ps. schmidlei* BDU 30313, and *D. baueriana* BDU 40481; in all these cases serum calcium level recorded lesser than the minimum of the normal range (8 mg%) (Fig-5 J). On the otherhand, with the strains *S. subsalsa* BDU 30311, *P. valderianum* BDU 20571 *P. tenue* BDU 41001 and *Lyngbya* sp. BDU 30601, serum calcium level increased to a level above the upper limit of the normal range (10.5 mg%). However, strains *S. subsalsa* BDU 30311, *O. formosa* BDU 40261, and *P. cortum* BDU 30241 did not affect the serum calcium level (Fig-5 M).

Calcium is a divalent cation circulating in the blood with in the range of 8.0 to 10.5 mg% as free calcium (Weisberg, 1977). Calcium is one of the physiologically important ions in the blood in that it is essential for irritability of the animal (muscular contraction and nervous conduction) (Nath, 1989). The changes in the blood calcium level of the treated rats don't appear to be a direct consequence of the calcium content of the cells of the various strains of cyanobacteria, because *D. baueriana* BDU 40481 and *Pl. terebrans* BDU 30342 are the ones in the present investigation containing the highest calcium content (Table-8) whereas, rats treated with the extract of these cyanobacteria, the calcium level decreased to below normal level. Similarly, those instances where the treatment resulted in increase in calcium level, the cyanobacteria viz.
P. valderianum and Lyngbya sp. BDU 30601 contain only moderate amounts of calcium (Table-8). Thus, it is apparent that the alteration in calcium levels brought about by the treatments was due to the impact of the cyanobacteria on the calcium homeostatic mechanism.

The homeostasis of calcium under the control of parathormone (PTH) and PTH is hypercalcemic. Vitamin D₃ is synergistic with PTH, enhancing the intestinal absorption of calcium. Calcitonin is hypocalcemic and works opposite to PTH (Gorbman et al., 1983). Therefore, those strains of cyanobacteria which tend to increase a blood calcium level should have stimulated the PTH-Vitamin D₃ system and those which tend to decrease the calcium level should have inhibited the system and activated the calcitonin mechanism.

4.3.10: SERUM MAGNESIUM

The magnesium content of the blood of the control rat was 4.56 ± 0.64 mg/100 mL. Excepting with P. tenue BDU 41001, where the magnesium of blood was maintained at normal level, in all other cases, the magnesium level increased, the highest value obtaining being with O. salina BDU 10142 (Fig-5 N).

Magnesium is another divalent cation whose metabolism is very closely related to that of calcium. It governs neuromuscular irritability and is important for activities of the coenzymes in metabolism of carbohydrates and proteins. The body store of magnesium is in the bone. In blood it is maintained at a constant level and any excess magnesium in circulation is excreted (Talwar and Bamezai, 1989). The
higher level of magnesium in the rats treated with the cyanobacterium probably reflects the magnesium content of this cyanobacterium, because dietary absorption of magnesium is correlated with the body requirement (Talwar and Bamezai, 1989).

4.3.11: SERUM SODIUM AND POTASSIUM

The control rats maintained serum sodium at 392 ppm and serum potassium at 19.5 ppm. Whereas, almost all the strains of cyanobacteria, tended to maintain near control serum sodium and potassium level, strains *S. subsalsa* BDU 30311 and *P. corium* BDU 30241 tended to decrease the serum sodium level, yet within the normal range; on the other hand strains *Sy. elongatus* BDU 30312 and *P. angustissimum* BDU 40061 tended to increase the serum potassium level and the strain *P. tenue* BDU 41001 decreased it; but the level deviated outside the normal range only with the strain *Sy. elongatus* BDU 30312 (Fig-5 K and L ).

Sodium and potassium are the two ions which are concerned with the osmoionic homeostasis, the third one being chloride ions. The normal range of sodium in human serum is 300 to 355 mg% and that of potassium is 14 to 22 mg% (Nair, 1989). Rats also maintain almost similar levels (Ravichandran and Selvam, 1991). The observation that with regard to sodium ion, the cyanobacteria do not alter it considerably, suggest that, the strains do not affect the osmo-ionic equilibrium of the animals. However, a special mention needs to be made about *Sy. elongatus* BDU 30312, the administration of which resulted is higher
serum potassium level, outside the normal range. However, this strain
did not affect the serum sodium level and therefore its impact on the
serum potassium level is not understandable.

4.3.12: SERUM GLUTAMINE OXALACETATE TRANSAMINASE (GOT)
AND GULTAMINE PYRUVATE TRANSAMINASE (GPT)

The activity of GOT (ALT) recorded in the control rats was
94.0 ± 14.35 U/mL, and GPT (AST) 57.60 ± 3.58 U/L. GOT level was
significantly increased on treatment with strain Sy. elongatus BDU
30312, O. formosa BDU 40261, P. angustissimum BDU 40061, Lyngbya
sp. BDU 30601 and Pl. terebrans BDU 30342 and decreased with S.
subsalsa BDU 30311, O. salina BDU 10142, and P. valderianum BDU
20571. GPT level was significantly increased in the case of strains
Sy. elongatus, BDU 30312 P. angustissimum BDU 40061, Lyngbya sp., BDU
30601, Pl. terebrans BDU 30342 and D. baueriana BDU 40481 and
decreased with strains O. salina BDU 10142 and P. tenue BDU
41001(Fig-5 O and P).

GOT is an enzyme that catalyses the reversible transfer of
the amino group from glutamine to oxalacetic acid. GPT catalyses the
reversible transfer of an amino group from glutamate to pyruvate
(Malaviya, 1989). These two are principally intracellular enzymes and
they are found in very minimal quantities in the serum (Oser, 1965).
GOT is found in cardiac, hepatic, skeletal muscle, renal and cerebral
tissue in decreasing concentrations. On the other hand, GPT has high
hepatic content compared to the relatively low concentration in
myocardial and other tissues (Malaviya, 1989). Damage in any form to the heart and liver causes the release of GOT into serum. On the other hand damage to the liver alone results in elevation of GPT to dramatic levels, and also GOT to a smaller extent; but the rise of GPT occurs sooner or faster and higher than GOT (Malaviya, 1989). Therefore, the activity level of GOT in blood is a diagnostic tool to infer damage to cardiac tissue. Elevation of serum GPT as well as GOT is taken to infer hepatic damage. It is also interesting to note that the normal ratio of GPT and GOT is 1:3 per L (Oser, 1965).

Analysis of the result of the present investigation in the above background suggests that treatment with strain Sy elongatus BDU 30312, Lyngbya sp. BDU 30601 and Pl. terebrens BDU 30342 were likely to have caused hepatocellular damage because of the elevated activity level of serum GOT and serum GPT. Probably these strains of cyanobacteria, contained a toxic substance which affect the liver function. In this context it is pertinent to point out the report(s) of the occurrence of some toxic substances in Lyngbya BDU 30601 (Richmond, 1988). On the other hand, in elevating serum activity of GOT, O. Jormosa BDU 40261 probably contains a toxin which affects the heart. Strains P. cortum BDU 30241 and D. baueriana BDU 40481 have affected specifically the activity level of GPT and therefore, probably affect the liver function.

However, one encouraging observation is that in none of the treatments the activities of either of the enzymes increased to above 500 units which is toxic at pathological level (Malaviya, 1989). However,
future histopathological analysis can alone throw further light in this regard.

4.3.13: SERUM ALKALINE (ALP) AND ACID PHOSPHATASES (ACP):

The serum acid phosphatase activity in control rats was 7.25 ± 1.92 KA units and alkaline phosphatase activity was 30.20 ± 4.7 KA units. The ACPase activity increased significantly on treatment with *P. angustissimum* BDU 40061, *P. corium* BDU 30241, *Pl. terebrans* BDU 30342 and *D. baueriana* BDU 40481 and it decreased with the strains *Sy. elongatus* BDU 30312 and *O. salina* BDU 10142. Strains *S. subsalsa* BDU 30311, *O. formosa*, *P. valderianum* BDU 20571, *P. tenue*, *Lyngbya* sp. BDU 30601 and *Ps. schmidlei* BDU 30313 did not affect the level of ACPase activity (Fig-5 Q and R). With regard to the ALPase, strains *Sy. elongatus* BDU 30312, *O. formosa* BDU 40261, *P. valderianum* BDU 20571, *P. angustissimum* BDU 40061, *P. tenue* BDU 41001, *Pl. terebrans* BDU 30342, *Ps. schmidlei* and *D. baueriana* BDU 40481, the activity increased and with *S. subsalsa* BDU 30311 and *Lyngbya* sp. BDU 30601, the activity decreased with strains *O. salina* BDU 10142 and *P. corium* BDU 30241, ALPase activity was not altered (Fig-5 Q and R).

Acid and alkaline phosphatases are nonspecific phosphomonoester phosphatases. The enzymes act on a variety of organic phosphate esters. Normal blood contains these two enzymes. Normal ALPase activity in adult man is 4 to 17 KA unit per 100 mL and in children 17 to 33 KA units and that of serum ACPase activity is about 0.5 to 4 KA unit per 100 mL (Malaviya, 1989). It has been said that
deviation of ALPase to 10 to 12 times the upper limit of normal is associated with the inhibition of bile flow, 2 to 5 times of the upper limit of normal in the case of failure of biliary canalicoli, 10 to 25 times the upper limit of normal in bone diseases and mildly increased in hyperparathyroidism (Malaviya, 1989). Increase in serum acid phosphatase activity is of concern only in the disease of prostatic carcinoma, where the level becomes alarming (Malaviya, 1989). Therefore, the changes in the serum acid phosphatase activity observed in the present study do not appear to be of much physiological significance. The alteration in ALPase activity, particularly on the higher side in several cases, appears to be within the normal range and the very high levels noticed in instances with the hypercalcimia, like in the case of P. valderianum BDU 20571 and P. tenue BDU 41001, suggesting demineralization of bone to mobilize calcium to blood (Turner and Bagnara, 1976).

4.4: CONCLUSIONS

Table-9 presents a summary of the very important bioactivities of the various marine cyanobacteria investigated in the present study, in the animal system, with probable application in the human system. Special mention needs to be made about S. subsalsa BDU 30311 whose richness in protein is reflected in the better conversion efficiency of the treated rats, O. formosa BDU 40261 in stimulating the blood clotting mechanism, P. valderianum BDU 20571 and P. angustissimum BDU 40061 in increasing conversion efficiency and Lyngbya sp. BDU 30601 and P. schmidlei BDU 30313 in
<table>
<thead>
<tr>
<th>S.No.</th>
<th>Organism</th>
<th>Beneficial effects</th>
<th>Risk factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Synechococcus elongatus</em></td>
<td>Useful in treatment for anaemia; carbohydrate supplement; decreases blood cholesterol level; hypocalcimic.</td>
<td>Increases RBC count; blood clotting mechanism affected; hypocalcimia; increased serum K level; possible heptacellular damage.</td>
</tr>
<tr>
<td>2</td>
<td><em>Spirulina subsalsa</em></td>
<td>Increases conversion efficiency; protein supplement; decreases blood cholesterol level.</td>
<td>Increases RBC count; blood clotting mechanism affected seriously.</td>
</tr>
<tr>
<td>3</td>
<td><em>Oscillatoria salina</em></td>
<td>Useful in the treatment for anaemia; carbohydrate supplement; decreases blood cholesterol level.</td>
<td>Increases RBC count; blood clotting mechanism affected; hypocalcimia; hyper-magnesimia.</td>
</tr>
<tr>
<td>4</td>
<td><em>Oscillatoria formosa</em></td>
<td>Blood clotting mechanism stimulated.</td>
<td>Decreased conversion efficiency; probably heart function.</td>
</tr>
<tr>
<td>5</td>
<td><em>Phormidium valderianum</em></td>
<td>Contributes to conversion efficiency; carbohydrate supplement; decreases cholesterol level; hypercalcimia.</td>
<td>Hyperglycemic.</td>
</tr>
<tr>
<td>6</td>
<td><em>Phormidium angustissimum</em></td>
<td>Better conversion efficiency</td>
<td>Hypocalcimia.</td>
</tr>
<tr>
<td>7</td>
<td><em>Phormidium corium</em></td>
<td>Useful in treatment for polycythemia; carbohydrate supplement.</td>
<td>Erythropoiesis affected probably liver function</td>
</tr>
</tbody>
</table>
Table-9 (Contd.):

<table>
<thead>
<tr>
<th>No.</th>
<th>Species</th>
<th>Effects</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.</td>
<td><em>Phormidium tenue</em> BDU 41001</td>
<td>Useful in treatment for anaemia; decreases blood cholesterol; hypercalcemia</td>
<td>Increases RBC count; blood clotting mechanism affected.</td>
</tr>
<tr>
<td>9.</td>
<td><em>Lyngbya sp.</em> BDU 30601</td>
<td>Useful in treatment for anaemia; blood clotting mechanism stimulated; insulin like effect; hypercalcemia.</td>
<td>Feed deterrent; negative growth; possible hepatocellular damage.</td>
</tr>
<tr>
<td>10.</td>
<td><em>Plectonema terebrans</em> BDU 30342</td>
<td>Useful in treatment for polycythemia.</td>
<td>Erythropoiesis affected; clotting mechanism affected; hypocalcemia; possible hepatocellular damage.</td>
</tr>
<tr>
<td>11.</td>
<td><em>Pseudoanabaena schmidtii</em> BDU 30313</td>
<td>Useful in treatment for anaemia; insulin-like effect; decreases blood cholesterol level.</td>
<td>Feed deterrent; negative growth; increases RBC count; clotting mechanism affected; hypocalcemia.</td>
</tr>
<tr>
<td>12.</td>
<td><em>Dichothrix baueriana</em> BDU 40481</td>
<td>Useful in treatment for anaemia.</td>
<td>Increases RBC count; hypocalcemia; liver function probably affected.</td>
</tr>
</tbody>
</table>
their insulin-like effect. In applying these strains special care has to be taken in using S. subsalsa BDU 30311 in view of the effect on the blood clotting mechanism and Lyngbya BDU 30601 in view of the toxin it reportedly contains.