4.0. CHAPTER 2

ANTIDIABETIC ACTIVITY OF S. POLYCYSTUM SODIUM ALGINATE ON ALLOXAN INDUCED DIABETIC RATS

4.1. INTRODUCTION

Diabetes is the most significant chronic disease and cause of death in modern society (Stratmann et al., 2007). It is a complicated metabolic disorder characterized by high blood glucose level due to inability of the body cells to utilize glucose properly (Ugochukwu and Babady, 2002). It is also increasing prevalence puts a large burden on society and the public health sector (Leroith and Smith, 2005). Type 1 diabetic is characterized by an absolute deficiency of insulin secretion, associated with autoimmune destruction of pancreatic β-cells, and this disease is more likely to occur in relatives of an affected person (Bottini et al., 2006). Type 2 diabetes, which accounts for more than 90% of cases, is caused by a combination of resistance to insulin action and impaired insulin secretion (Warren, 2004).

Diabetes is a multidimensional disorder and its management needs firm adherence to the prescribed treatment plan. The contemporary treatment of diabetes is focused on suppressing and controlling blood glucose to a normal level. The common agreement on management of type 2 diabetes is change in lifestyle along with appropriate diet and weight control. However, antidiabetic drugs are needed as these measures cannot provide satisfactory results. Antidiabetic drug therapy includes insulin
injections and oral hypoglycemic drugs. These drugs act by various mechanisms to control the blood glucose level. However, many side-effects such as hypoglycemia, lactic acid intoxication and gastrointestinal upset, etc. have been reported in diabetic patients, who undergone treatment with antidiabetic allopathic drugs (Li et al., 2004). Because the antidiabetic medication may sometimes involve prescribing more than one drug at the same time, which can augment the severity of these side-effects, efforts are being made to find a suitable antidiabetic therapy (Khan et al., 2012).

The plant kingdom is a wide field to search for natural effective oral antidiabetic agents with slight or no side effects. In experimental studies, several medicinal herb extracts already showed significant hypoglycemic or hypolipidemic properties (Bailey and Day, 1989; Mentreddy, 2007; Frode and Medeiros, 2008). Dewanjee et al. (2009) have reported the antidiabetic potential of swietenine from *Swietenia macrophylla* seeds in neonatal-streptozotocin (STZ) induced diabetic rats through oral administration. Bhaskar et al. (2008) studied the hypoglycemic effect of *Mucuna pruriens* seed extract on normal and streptozotocin-diabetic rats through oral administration. Kannur et al. (2006) investigated the antidiabetic activity of *Caesalpinia bonducella* seed extracts in alloxan induced diabetic rats. Maiti et al. (2005) pointed out that the aqueous extract of seeds of the medicinal plant *Tamarindus indica* (Caesalpiniaceae) has got better antidiabetic effect against STZ induced diabetic mice. Kumar et al. (2008) have also stated that the antidiabetic potential of *Phyllanthus reticulates* in alloxan induced diabetic mice.
Recently, some fibre products, extracted from seaweeds are used for the treatment of diabetics. The polyuronic saccharide sodium alginate is a fibre product isolated from the cell wall of brown algae and some bacteria, which has many advantages on treating diabetics (Brownlee et al., 2005). Another natural product alginites have wide range of applications in the food and pharmaceutical sectors (Strugala et al., 2004). Alginate is capable of forming a cross-linked gel network in the presence of divalent cations such as calcium. To date, there are few studies conducted on the effect of sodium alginate on lipemia and glycemia. There is an inverse linear relationship between supplementary dietary fibre intake and plasma cholesterol for alginate and an associated increase in total faecal bile levels (Seal and Mathers, 2001). Kimura et al. (1996) examined a range of alginate of Lessonia angustata Kjellman vat. longissima formulations in rats, showing increased cholesterol excretion and improved glucose tolerance. Wolf et al. (2002) demonstrated a fall in peak glycemia after ingestion of a viscous alginate drink. When the sodium alginate is orally administered, it is converted to free alginic acid in the stomach; however, free alginic acid is not absorbed from the small intestine, since mammals have no enzyme capable of digesting alginic acid. Sodium alginate is reported to be effective in enhancing the excretion of cholesterol (Keys et al., 1961; Tsuji et al., 1968 and 1977) and in suppressing the biological absorption of harmful metals such as strontium and cadmium (Hesp and Ramsbottom, 1965; Sutton, 1967; Kojima et al., 1980; Kai et al., 1982). Thus, it seems likely that sodium alginate functions as a dietary fibre and it is widely used to prevent hyperlipemia and obesity. Further, an aqueous 5% solution of sodium alginate has been
used in the treatment of peptic ulcers and esophagitis (Daigo et al., 1981a, b). Considering the importance of the above findings, the present study is proposed to find out the antidiabetic effect of sodium alginate extracted from brown seaweed *S. polycystum* on alloxan induced diabetic rats with the following objectives.

**Objectives**

1. To induce diabetes by single intraperitoneal injection of alloxan in Wistar albino rats.
2. To determine the blood glucose level after feeding with different concentrations of sodium alginate through oral administration for 45 days.
3. To record the growth performance of control and experimental rats after 45 days of feeding experiment.
4. To analyze the hematological parameters, blood serum lipid profile, kidney function test, key enzyme activities of blood and kidney, reducing sugar and reducing cholesterol level of the experimental rats at the end of 45th day of feeding experiment.
4.2. MATERIALS AND METHODS

4.2.1. Experimental animal

Male Wistar albino rats (150 - 180g) were chosen for the present study (Plate 4.1). All the rats were kept at room temperature (28 ± 1°C) in the animal house. Animal handling and experimental procedures were approved by the Institutional Animal Ethics Committee, Centre for Marine Science and Technology, Manonmaniam Sundaranar University, Rajakkamangalam, Kanyakumari District, Tamilnadu and the animals were cared in accordance with the “Guide for the care and use of laboratory animals” (NIH, 1985) and “Committee for the purpose of control and supervision on experimental animals” (CPCSEA, 2004). The rats were maintained on commercial food pellets and water at ad libitum.

4.2.2. Acute oral toxicity study

The acute oral toxicity study of \textit{S. polycystum} - sodium alginate was carried out by adopting limit test method as per the OECD (Organisation for Economic Co-operation and Development) guidelines 425, adopted on 17\textsuperscript{th} December 2001 (OECD, 2001). Female nulliparous and non-pregnant adult Wistar rats (150-180g) were used for this study. They were allowed to acclimatize for seven days to the laboratory condition before the experiment and were maintained on standard animal diet and provided with water \textit{ad libitum}. Later they were randomly distributed in to one control group and another treatment group containing six animals per group. The animals were kept fasting for overnight and provided only water, prior to compound administration, after
which the body weight of animals were determined. A single test dose of 1250mg/kg of *S. polycystum* sodium alginate in 2ml of 0.1M phosphate buffered saline was given to the treatment group by gastric intubations. The control group received 2 ml of 0.1M phosphate buffered saline alone. The animals were observed continuously for the first 4h and for every one hour interval for the next 24 h, then 6 h intervals for 2 days after administering sodium alginate to observe any changes in general behaviour, physiological activities and mortality up to 14 days. The parameters observed were grooming, hyperactivity, diarrhea, sedation, respiratory distress, abnormal locomotion, tremors and convulsions (Shah Ayub *et al.*, 1997; Borger *et al.*, 2005).

4.2.3. Hypoglycemic activity

Male albino rats were made diabetics with a single dose (120mg/kg) of Alloxan (Central Drug House (p) Ltd., New Delhi) by intraperitoneal route (Joy and Kuttan, 1999). After 3 days of administration, diabetics was confirmed in all the rats by the determination of fasting blood glucose concentration (>250mg/dl) by digital glucometer (One tough ultra glucometer). Then these rats were divided into 7 groups of 6 numbers each and treated orally as follows.

Group 1- Normal control, given only saline (10ml/kg, once a day, daily).

Group 2- Diabetic control - alloxan induced diabetic given only saline (10ml/kg, once a day, daily).

Group 3- Alloxan induced diabetic rats treated with sodium alginate of *S. polycystum* (250mg/kg, orally, once a day, daily).
Group 4- Alloxan induced diabetic rats treated with sodium alginate of *S. polycystum* (500mg/kg, orally, once a day, daily).

Group 5- Alloxan induced diabetic rats treated with sodium alginate of *S. polycystum* (750mg/kg, orally, once a day, daily).

Group 6- Alloxan induced diabetic rats treated with sodium alginate of *S. polycystum* (1000mg/kg, orally, once a day, daily)

Group 7- Alloxan induced diabetic rats treated with sodium alginate of *S. polycystum* (1250mg/kg, orally, once a day, daily)

After this treatment, the blood glucose level was measured at an every 3 days intervals up to 45 days of experiment by using digital glucometer. During each treatment, all the rats were fed with commercial pellet feed (Sai enterprise, Chennai) (Plates 4.2 to 4.6).

4.2.4. Growth studies

At the end of the experiment (45th day), the growth (either weight loss or gain) was determined by measuring the weight of control and experimental rats of each group. The weight gain / loss were calculated by deducting the initial weight from the final weight.

4.2.5. Collection and processing of blood and tissue samples

4.2.5.1. Blood samples

At the end of the treatment (45th day), the individual groups of rats were sacrificed by cervical dislocation, blood samples were collected individually in tubes
with ethylene diamine tetra acetic acid (EDTA) by direct cardiac puncture and was allowed to coagulate at ambient temperature for 40min. From this, serum was separated by centrifugation at 2000rpm for 10min and used for the analysis of hematological and biochemical parameters (Plate 4.7).

4.2.5.2. Tissue samples

The tissues such as liver and kidney were dissected out individually from each group of rats, washed with cold physiological saline, and washed with distilled water to remove adherent lipids and immediately transferred into ice-cold containers (Plate 4.8 and 4.9). The liver and kidney (250mg each) tissues were individually sliced into pieces and homogenised in appropriate buffer (pH 6-8) in cold condition to 20% homogenate (w/v). The homogenates were centrifuged individually at 3000rpm for 10 min at 0°C in a refrigerated centrifuge. The supernatant was separated and used for various biochemical estimations. The pancreatic tissue was also dissected out from each group of rats individually and immediately preserved in 10% formaldehyde solution for histopathological study.

4.2.6. Analysis of blood hematological parameters

4.2.6.1. Red blood cells count (Erythrocytes)

The mature erythrocyte is a biconcave disk, circular shape, centrally unstained and periphery stained, pink colour. Size: 7.2 microns in average diameter. It contains haemoglobin.
RBC Diluting fluid

(a) Formal Citrate Solution
- Trisodium citrate : 3g
- Distilled water : 99ml
- Formalin (Commercial) : 1ml

(b) Hayem’s fluid
- Sodium chloride : 0.5g
- Sodium sulphate : 2.5g
- Mercuric chloride : 0.25g
- Distilled water : 100ml

The blood samples drawn from the experimental rats were individually pipetted using RBC pipette and immediately added with the diluting fluid (Formal Citrate solution or Hayem’s fluid) to the mark 101, and rotated the pipette between the thumb and forefinger. This gave a dilution of 1:200.

In a clean counting chamber, a cover glass was placed in position over the ruled area, using gentle pressure. Mixed the suspension thoroughly by rotating the pipette for about a minute, holding it in horizontal position, and finally shaken sidewise. Expelled the fluid from the stem of the pipette without loss of time, filled the chamber by holding the pipette at an angle of 45° and slightly touching the tip against the edges of the cover glass. Care was taken to ensure that the suspension did not flow on either sides or any
bubble form under cover glass. Allowed two to three minutes for the red blood corpuscles to settle.

The number of RBC’s in 80 small squares (4 squares of 16 at the four corners and one of 16 at centre) were counted. But the cells touching the lower and right hand lines, out count the cells touching the upper and left hand lines were not counted.

4.2.6.2. White Blood Cells count (Leucocytes)

**WBC Diluting Fluid (Truck’s)**

- Acetic acid (glacial) : 3ml
- Distilled water : 97ml

(Added Gention violet to get a pale violet colour)

The blood was drawn upto the 0.5 mark in WBC pipette and diluted up to the mark 11 with WBC fluid as described in RBC counting and filled the counting (Neubauer) chamber in the same manner and allowed 3 min for cells to settle. The cells were counted in the four corner blocks of the counting chamber. When counting the cells, those cells touching on the inner lines on the right and top were counted, but the cells did not touching the lines on the left and bottom were not counted. The difference between the two square millimeter areas should not be more than 10 WBC’s.

4.2.6.3. Haemoglobin estimation

Haemoglobin in the blood samples was estimated by the method of Drabkin and Austin (1932). To 0.02ml of blood, 5 ml of Drabkin’s reagent was added, mixed well
and allowed standing for 10 min. The colour developed was read at 540nm in a spectrophotometer together with the standard reagent against a reagent blank.

**Calculation**

\[
\text{Haemoglobin (g/dl)} = \frac{\text{OD of the test}}{\text{OD of the standard}} \times \text{Concentration of the standard}
\]

### 4.2.6.4. Estimation of glycosylated haemoglobin (HbA\textsubscript{1c})

Glycosylated haemoglobin in the blood samples of individual group of rats was estimated by the method of Sudhakar Nayak and Pattabiraman (1981). 0.5ml of saline washed erythrocytes were lysed with 5.5ml of water, mixed and incubated at 37°C for 15 min. The contents were centrifuged and the supernatant was discarded, then 0.5ml of saline was added, mixed and processed for estimation. To 0.2ml of aliquot, 4ml of oxalate hydrochloric solution was added and mixed. The contents were heated at 100°C for 4h, cooled and precipitated with 2ml of 40% TCA. The mixture was centrifuged and to 0.5ml of supernatant, 0.05ml of 80% phenol and 3.0ml of concentrated sulphuric acid were added. The colour developed was read at 480nm in a spectrophotometer after 30min.

**Calculation**

\[
\text{HbA}_{1c} \text{(mg/g Hb)} = \frac{\text{OD of the test}}{\text{OD of the std.}} \times \frac{\text{Conc. of the std}}{\text{Vol. of aliquot used}} \times \frac{\text{Total vol. of aliquot}}{\text{Vol. of blood}} \times \frac{1}{\text{Hb(g)}}
\]
4.2.7. Analysis of blood serum lipid profile

4.2.7.1. Estimation of Total cholesterol

Total cholesterol level in the serum of control and experimental rats was estimated by the enzymatic method described by Allain et al. (1974). To 10µl of serum, 1ml of enzyme reagent was added, mixed well and kept at 37°C for 5 min. 10µl of cholesterol standard and distilled water (blank) were also processed similarly. The absorbance was measured at 510nm by using spectrophotometer.

**Calculation**

\[
\text{Total Cholesterol (mg/dl)} = \frac{\text{OD of the test}}{\text{OD of the standard}} \times \text{Concentration of the standard}
\]

4.2.7.2. Estimation of triglycerides

Triglyceride level in the serum was estimated using the diagnostic kit based on the enzymatic method described by McGowan et al. (1983). To 10µl of serum, 1 ml of enzyme reagent was added, mixed well and incubated at room temperature for 10 min. 10µl of triglyceride standard and distilled water (blank) were also processed similarly. The absorbance was measured at 510nm by using spectrophotometer.

**Calculation**

\[
\text{Triglycerides (mg/dl)} = \frac{\text{OD of the test}}{\text{OD of the standard}} \times \text{Concentration of the standard}
\]
4.2.7.3. Estimation of HDL- Cholesterol (HDL-C)

HDL cholesterol in serum was estimated by using the diagnostic kit based on the enzymatic method of Izzo et al. (1981). 0.1ml of blood serum was mixed with 0.1ml of precipitating reagent, allowed to stand at room temperature for 5min and centrifuged at 2000-3000 rpm for 10 min. Cholesterol in the clear supernatant was estimated as per the procedure described earlier (Section 4.2.7.1.).

**Calculation**

\[
\text{HDL-C (mg/dl)} = \frac{\text{OD of the test}}{\text{OD of the standard}} \times \text{Concentration of the standard}
\]

4.2.7.4. Estimation of VLDL cholesterol (VLDL-C) and LDL cholesterol (LDL-C)

VLDL-C and LDL-C in serum were calculated by the method of Friedewald et al. (1972).

The formula used were,

\[
\text{VLDL-C} = \frac{\text{Triglycerides}}{5}
\]

\[
\text{LDL-C} = \text{Total cholesterol} - (\text{HDL-C} + \text{VLDL-C})
\]

The values were expressed as mg/dl of plasma.

4.2.8. Kidney function tests

4.2.8.1. Blood urea

Blood urea was estimated by the method of Natelson et al. (1951). To 0.1ml of blood pipetted in a test tube, added 3.3ml of water, 0.3ml of sodium tungstate and 0.3ml of 2/3N H\textsubscript{2}SO\textsubscript{4}, mixed well and centrifuged. From this 1ml of the supernatant was
pipetted out and added 1ml of water, 2ml of di-acetyl monoxime (DAM) reagent and 2ml of urea acid reagent. The tubes were kept in boiling water bath for 30min, cooled and the pink colour developed was read at 480nm against a reagent blank containing 2ml of water, 2ml of DAM and 2ml of the urea acid reagent. For a standard curve, 0.4 to 2ml of urea working standards of concentrations 0.02 to 0.1mg in different test tubes were treated in similar way as the test.

**Calculation**

\[
\text{Urea (mg/dl)} = \frac{\text{OD of the test}}{\text{OD of the std.}} \times \frac{\text{Con. of the std.}}{\text{Vol. of supernatant}} \times \frac{\text{Total vol. of supernatant}}{\text{Vol. of blood}} \times 100
\]

4.2.8.2. Blood serum creatinine

**Principle**

In alkaline medium, Picric acid reacts with creatinine to produce a red coloured complex and intensity of which is measured at 540 nm (or using a green filter).

**Reagents**

1. Tungstic acid reagent (established)
2. Creatinine reagent
3. Sodium hydroxide reagent (1.4N)
4. Creatinine standard (12 mg)
**Procedure**

**Protein free solution**

Protein free solution was prepared by taking 4.5 ml of tungstic acid reagent in a centrifuge tube and 0.5 ml of serum was added. Then it was mixed well, allowed to stand for 5 min and centrifuged.

3 tubes were taken and marked as test (T), standard (S) and Blank (B). 1 ml each of creatinine reagent was added in to each tube. Simultaneously 3 ml each of distilled water was added to standard and blank tubes. 0.1 ml of creatinine standard was added in standard tube and mixed well. Then 3ml of protein free solution and 0.5ml of sodium hydroxide reagent were added in to all the above tubes and mixed well. The mixture was incubated at room temperature for 5 min. The OD was measured at 540nm in spectrophotometer.

**Calculation**

\[
\text{Mg creatinine per 100ml serum} = \frac{\text{OD of test} - \text{OD of blank}}{\text{OD of std} - \text{OD of blank}} \times 4
\]

**4.2.8.3. Uric acid**

**Reduction of alkaline phosphotungstate**

**Principle**

Uric acid reduces phosphotungstate to tunsten blue color of which is measured at 650 nm (using a red filter).
Reagents

(i) Tunstic acid reagent (stablished)
(ii) Phosphotungstic acid reagent
(iii) Sodium carbonate (10%)
(iv) Uric acid standard (75 mg)

Procedure

Protein free solution

Protein free solution was prepared by taking 4.5 ml of tungstic acid reagent in a centrifuge tube and 0.5 ml of blood serum was added. Then it was mixed well, allowed to stand for 5 min and centrifuged.

3 tubes were taken and marked as test (T), standard (S) and Blank (B). Then 3ml of protein free filtrate was added to test tube. 3ml each of distilled water was added to the standard and blank test tubes. 0.02 ml of uric acid standard was added to the standard tube. Then 1ml of sodium carbonate solution and 1ml of phosphotungstic acid reagent were added in to each tube and mixed well. The mixture was incubated for 5min at room temperature. The OD was measured at 650nm in a spectrophotometer.

Calculation

Mg Uric acid per 100 ml serum = \( \frac{\text{OD of test} - \text{OD of blank}}{\text{OD of std} - \text{OD of blank}} \times 5 \)
4.2.9. Analysis of key enzymes of carbohydrate metabolism in liver and blood serum samples

4.2.9.1. Assay of glycogen phosphorylase in liver samples

Glycogen phosphorylase was assayed by the method of Sutherland et al. (1957). 250mg of chilled liver tissue was homogenised at 0°C with 5ml citrate buffer (pH 6), centrifuged at 3000rpm for 10 min at 0°C and 0.5ml of the supernatant was used for the assay. Assay medium containing 0.6ml of sodium fluoride, 0.2 ml of glucose-1-phosphate, 0.6ml of citrate buffer, 0.1 ml of 4% glycogen and 0.5ml of the enzyme source were kept for 30 min at 30°C. Then 1ml of 10% TCA was added, diluted to 10ml using 7ml of distilled water. It was then centrifuged and 1ml of supernatant was used for phosphate estimation by the method described by Fiske and Subbarow (1925). To 1ml of the supernatant, 0.6ml of distilled water, 1ml of ammonium molybdate and 0.4ml of ANSA reagent were added. The blue colour developed after 20min was then read against a reagent blank at 620nm. Tubes containing 0.5 to 2.5 ml of the working standard of concentrations 4 to 20µg were also treated in the same way as the test.

**Calculation**

\[
\text{Glycogen phosphorylase (µ moles of phosphorus liberated/h/mg protein) = } \frac{\mu \text{ moles of phosphorus liberated}}{\text{Vol. of supernatant} \times \text{Total volume}} \times \frac{1}{\text{Vol. of enzyme extract}} \times \frac{60}{\text{Incubation time}} \times \frac{1}{\text{mg protein}}
\]
4.2.9.2. Assay of hexokinase in liver samples

Hexokinase was assayed by the method of Brandstrup et al. (1957). 250mg of the chilled liver tissue was homogenised at 0°C with 0.01M Tris-HCl buffer at pH 8 (1:1 w/v), centrifuged at 3000rpm for 10min at 0°C and 1.0ml of the supernatant was used for the assay. The reaction mixture in a total volume of 5ml contained the following: 1ml of glucose solution, 0.5ml of magnesium chloride, 0.5ml of dipotassium hydrogen phosphate solution, 0.4 ml of potassium chloride, 0.1ml of sodium fluoride solution and 2.5ml of Tris HCl buffer (pH 8). The mixture was pre incubated at 37°C for 5min. The reaction was initiated by the addition of 1ml of enzyme source. Immediately (zero time), 1.0ml aliquot of the reaction mixture was taken in a tube containing 1ml of 10% TCA. A second aliquot was removed after 30min of incubation at 37°C and added to a tube containing 1ml of 10% TCA. The precipitated protein was removed by centrifugation and the residual glucose in the supernatant was estimated by the o-toluidine method of Sasaki and Senae (1972). The supernatant was mixed with 4ml of o-toluidine reagent and was kept in boiling water bath for 15 min. The green colour developed was read colorimetrically at 620nm. A reagent blank was run with each test. The amount of glucose phosphorylated was given by the difference between the two values.

Calculation

\[
\text{Hexokinase (µ moles of glucose phosphorylated /h/mg protein) = } \frac{\mu \text{ moles of glucose phosphorylated}}{\text{Vol. of aliquot used}} \times \frac{\text{Total volume of aliquot}}{\text{Incubation time}} \times \frac{60}{\text{mg protein}}
\]
4.2.9.3. Assay of glucose 6-phosphatase in liver samples

Glucose 6-phosphatase was assayed by the method of Koide and Oda (1959). 250mg of the chilled liver tissue was homogenised at 0°C with 0.1M citrate buffer at pH 6 (1:1 w/v), centrifuged at 3000rpm for 10 min at 0°C and 0.2 ml of the supernatant was used for the assay. Pipetted out 0.3ml of citrate buffer, 0.5ml of glucose 6-phosphate and 0.2ml of tissue homogenate in a test tube and incubated at 37°C for 1h. 1ml of 10% TCA was added to the tube to terminate the enzyme activity and then centrifuged. The phosphate content of the supernatant was then estimated by the method of Fiske and Subbarow (1925). To 1ml of the aliquot of supernatant, 1ml of ammonium molybdate and 0.4ml of ANSA reagent were added. The blue colour developed was read after 20min at 620nm. A tube devoid of the enzyme was served as control. A series of standard containing 8-40 µg of phosphorus was treated similar along with a blank containing only the reagent.

Calculation

Glucose 6-phosphatase (µ moles of inorganic phosphate liberated/min/mg.protein) =

\[
\frac{\text{µ moles of inorganic phosphate liberated}}{\text{Vol. of tissue homogenate}} \times \frac{1}{60} \times \frac{1}{\text{mg protein}}
\]

4.2.9.4. Assay of fructose 1,6-bisphosphatase in liver samples

Fructose 1,6-bisphosphatase was assayed by the method of Gancedo and Gancedo (1971). 250mg of the chilled liver tissue was homogenised at 0°C with 0.1M Tris HCl buffer at pH 7 (1:1 w/v), centrifuged at 3000rpm for 10min at 0°C and 0.2ml
of the supernatant was used for the assay. The assay medium in a final volume of 2ml contained 1ml of Tris-HCl buffer, 0.4ml of substrate, 0.1ml of magnesium chloride, 0.2ml of potassium chloride, 0.1ml of EDTA and 0.2ml of enzyme source. The tube was incubated at 37°C for 15min. The reaction was then terminated by the addition of 1ml of 10% TCA. The suspension was centrifuged and the phosphorus content of the supernatant was estimated according to the method described by Fiske and Subbarow (1925). To 1ml of an aliquot of the supernatant, 0.3ml of distilled water and 0.5ml of ammonium molybdate were added. After 10min, 0.2ml of ANSA reagent was added. The tubes were shaken well, kept aside for 20min and the blue colour developed was read at 620nm. Tubes containing 0.5 to 2.5ml of the working standard of concentrations 4 to 20µg were also treated in the same way as the test.

**Calculation**

Fructose 1,6-bisphosphate (µ moles of inorganic phosphorus/h/mg protein) =

\[
\frac{\mu \text{ moles of inorganic phosphorus liberated}}{\text{Vol. of supernatant}} \times \frac{\text{Total Volume}}{\text{Vol. of enzyme extract}} \times \frac{1}{\text{Incubation time}} \times \frac{60}{\text{mg protein}}
\]

**4.2.9.5. Assay of alanine transaminase (ALT) in blood serum samples**

The activity of alanine transaminase (ALT) was determined by the method of Mohur and Cook (1957).
Reagents

(i) Buffered substrate solution (0.1 M phosphate buffer (pH 7.4), 0.2 M DL alanine, 2.0 mM 2-oxoglutarate): 1.5g dipotassium hydrogen phosphate, 0.2g potassium dihydrogen phosphate, 0.03g 2-oxoglutaric acid and 1.78g DL alanine were dissolved in distilled water. The pH was adjusted to 7.4 with 1N NaOH and made up to 100 ml.

(ii) 20mg 2,4 dinitrophenyl hydrazine (DNPH) in 100 ml of hot 1N hydrochloric acid.

(iii) 0.4N Sodium hydroxide.

(iv) Standard pyruvic acid: 12.50 mg of sodium pyruvate was dissolved in 10 ml of distilled water. 10 ml of this was diluted to 100 ml with distilled water and this was prepared freshly for the calibration curve.

Procedure

To 1.0 ml of the buffered substrate, 0.1 ml of blood serum sample was added and incubated at 37°C for 30 min. The reaction was arrested by adding 1.0 ml of DNPH and left aside for 20 min at room temperature. Colour developed by the addition of 10 ml of 0.4N NaOH was read at 540nm in a UV spectrophotometer against the reagent blank.

Calculation

\[
ALT \ (IU) = \frac{\mu \text{ moles of pyruvate formed}}{\text{Vol. of blood serum}} \times \frac{1}{\text{Incubation time (min)}}
\]
4.2.9.6. Assay of aspartate aminotransferase (AST) in blood serum samples

The aspartate aminotransferase (AST) activity was assayed by the method of Mohur and Cook (1957).

Reagents

(i) Phosphate buffer: 0.15M (pH 7.5)

(ii) Substrate: 300mg of L-aspartic acid and 50mg of α-ketoglutaric acid were dissolved in 20-30 ml of the phosphate buffer and added 10% sodium hydroxide to bring the pH to 7.5 and was made up to 100 ml with phosphate buffer.

(iii) 2,4-dinitro phenyl hydrazine (DNPH) reagent: Dissolved 200mg of DNPH in 85 ml of concentrated hydrochloric acid and made up to a litre with distilled water.

(iv) 0.4 N sodium hydroxide.

(v) Standard pyruvic acid: 12.5mg of sodium pyruvate was dissolved in 10 ml of distilled water. 10 ml of this was diluted to 100 ml with distilled water and this was prepared freshly for the calibration curve.

Procedure

To 1.0 ml of the buffered substrate, 0.1 ml of the sample was added and incubated for one hour at 37°C. Then 1.0 ml of DNPH reagent was added and left for 20 min. At the end of incubation, 10 ml of 0.4 N NaOH was added and the colour developed was estimated by reading OD at 540nm in a UV spectrophotometer after 10 min. The standards were also treated similarly.
Calculation

\[
\text{AST (IU)} = \frac{\mu \text{ moles of pyruvate formed}}{\text{Vol. of serum}} \times \frac{1}{\text{Incubation time (min)}}
\]

4.2.9.7. Assay of Alkaline phosphatase (ALP) in blood serum samples

Alkaline phosphatase was assayed by the method of King (1965) using disodium phenyl phosphate as the substrate.

Reagents

(i) Carbonate-bicarbonate buffer (pH 10)

(ii) Substrate: 0.01M disodium phenyl phosphate solution.

(iii) Folin's phenol reagent: In a 1500 ml round-bottomed flask, 100 g of sodium molybdate, 700 ml water, 50 ml of 85% O-phosphoric acid and 100 ml of concentrated hydrochloric acid were added. The mixture was refluxed gently for 10h. Then, 150 g of lithium sulfate, 50 ml of water and few drops of bromine were added. The mixture was boiled for 15 min to remove excess bromine. The contents were cooled and diluted to one litre and filtered. This was diluted at 1:2 with double distilled water before use.

(iv) 15% sodium carbonate

(v) 0.1 M magnesium chloride

(vi) Standard phenol solution: A solution of distilled crystalline phenol in water, containing 5.0 g in 0.1 ml was prepared.
Procedure

The incubation mixture contained the following components in a final volume of 3.0 ml. 1.5 ml of carbonate-bicarbonate buffer, 1.0 ml of substrate and 0.1 ml of magnesium chloride and required amount of the enzyme source (0.2 ml serum). The reaction mixture was incubated at 37°C for 15 minutes. The reaction was terminated by the addition of 1.0 ml of Folin's phenol reagent. If turbidity appeared, the tubes were centrifuged. Controls without enzyme sources were also incubated and the enzyme source was added after the addition of Folin's phenol reagent. Then 1.0 ml of 15% sodium carbonate solution was added and incubated for further 10 min at 37°C. The blue colour developed was read at 640 nm in a UV spectrophotometer against a blank. The standards were also treated similarly.

Calculation

\[
\text{ALP (KA units)} = \frac{\text{mg phenol liberated}}{\text{Vol. of supernatant}} \times \frac{\text{Total volume of supernatant}}{\text{Vol. of serum}} \times 100
\]

4.2.9.8. Assay of Lactate dehydrogenate (LDH) in blood serum samples

The lactate dehydrogenase (LDH) activity was assayed according to the method of King (1965) with slight modification. The amount of pyruvate formed in the forward reaction was measured calorimetrically.

Reagents

(i) 0.1M glycine buffer: 7.5 g of glycine and 5.88 g of sodium chloride were dissolved in one litre of distilled water.
(ii) Buffered substrate: 2.76 g of lithium lactate was dissolved in 125 ml of glycine buffer containing 75 ml of 0.1 N sodium hydroxide to adjust the pH to 10. This was prepared just prior to use.

(iii) 0.4 N Sodium hydroxide.

(iv) 5.0 mg of NAD$^+$ was dissolved in 1.0 ml of distilled water. This was prepared just before use.

(v) 2,4-dinitrophenyl hydrazine (DNPH): 200 mg of DNPH was dissolved in one litre of 1 N HCl.

(vi) Standard pyruvate solution: 12.5 mg of sodium pyruvate was dissolved in 100 ml of buffered substrate.

**Procedure**

To 1.0 ml of the buffered substrate, 0.2 ml of the sample was added and the tubes were incubated at 37°C for 15 min. After adding 0.2 ml of NAD$^+$ solution, the incubation was continued for 30 min and then 1.0 ml of DNPH reagent was added. Then the tubes were incubated at 37°C for 15 min, followed by 7.0 ml of 0.4 N NaOH was added and the colour developed was measured at 540 nm in a UV spectrophotometer against the reagent blank. Suitable aliquots of the standards were also treated in the same manner.

**Calculation**

\[
LDH \text{ (IU/L)} = \frac{\mu \text{ moles of pyruvate formed}}{\text{Vol. of serum}} \times \frac{1}{\text{Incubation time (min)}
\]
4.2.10. Determination of reducing substances in fecal samples

4.2.10.1. Reducing sugar

2ml each of benedict solution was taken in 10ml test tubes and heated for 10 min. Then known amount of fecal samples of control and experimental rats were added individually in respective test tubes. Then the test tubes were shaken well and heated for 10 min. The following changes in colour were determined as the reducing sugar level.

Green colour: (0.5%); Yellow colour: (1.0%); Orange color: (1.5%); Red colour: (2.0%).

4.2.10.2. Reducing cholesterol

The reducing cholesterol level in the fecal samples of control and experimental rats was determined by the estimation of total cholesterol method described by Allain et al. (1974). To 10µl of fecal sample, 1ml of enzyme reagent was added, mixed well and kept at 37°C for 5 min. 10µl of cholesterol standard and distilled water (blank) were also processed similarly. The absorbance was measured at 510nm by using spectrophotometer.

Calculation

\[
\text{Total cholesterol in fecal (mg/dl)} = \frac{\text{OD of the test}}{\text{OD of the standard}} \times \text{Concentration of the standard}
\]

4.2.11. Histopathology of the pancreas

For histopathological study, rats from each group were sacrificed and the pancreatic tissue from individual group of rats were excised immediately, washed with
cold physiological saline (0.9% w/v) and fixed in 10% formalin (Plate 4.10). Then the tissues were dehydrated by treatment with a series of different concentrations of isopropyl alcohol and embedded in paraffin wax. 3-5µm thick sections were cut using a microtome and stained with haematoxylin and eosin. The specimens were evaluated with Phase Contrast microscope (Nikon Eclipse TS100, Japan) and photographed. All histopathological changes were examined with the help of a pathologist.

4.2.12. Statistical analysis

The data obtained in the present study were expressed as Mean ± SD and were analyzed using one way ANOVA at 5% significance level. Further a Tukey’s multiple comparison tests was conducted to compare the significant differences among the parameters using computer software Statistica 6.0 (Statsoft, UK).
4.3. RESULTS

The present study was carried out to determine the effect of *S. polycystum* sodium alginate on diabetic as well as diabetic induced metabolic disorders in diabetic rats. The detailed results are given below.

4.3.1. Acute oral toxicity study

During the acute toxicity study, the behaviour of sodium alginate treated Wistar albino rats appeared normal, and no toxic effect and mortality caused by the dose (1250mg/kg) of sodium alginate. From the result, it was understood that the test animals were found to be safe up to the dose of 1250 mg/kg sodium alginate and therefore, the doses of 250, 500, 750, 1000 and 1250mg/kg of sodium alginate were fixed for further studies.

4.3.2. Weight differences of control and experimental rats

The result on the difference in body weight of Wistar albino rats before and after the sodium alginate treatment is given in Table 4.1. The initial weight of control and experimental groups of rats was ranged between 160.50 and 175.38g. After 45 days of the experiment, the weight of all the groups of rats was varied much. For instance, the final weight of normal control rats, which received only the saline water increased to 169.40g. At the same time, the weight of diabetic control rats was drastically reduced to 149.24g. It was found to be 7.015% weight reduction from the initial weight. Similarly the change in weight of all the experimental groups of rats was also varied considerably
and it was related to the concentration dependent variation of sodium alginate. When the concentration level of sodium alginate increased, the weight of rats reduced positively. i.e at the lowest concentration (250 mg/kg) of sodium alginate, the weight loss from the initial weight was only 4.315%, whereas the rats treated with 500 to 1250mg/kg concentrations of sodium alginate showed the weight loss of 5.716, 6.716, 8.040 and 9.579%, respectively at the end of the experiment. The statistical one way ANOVA test revealed that the percentage weight loss of Wistar albino rats as a function of variation between diabetic control and different concentrations of sodium alginate treated groups was statistically more significant (F= 269.2627; P< 0.0001) (Table 4.1a).

4.3.3. Blood glucose level

The blood glucose level recorded in the male Wistar albino rats before and after the sodium alginate treatment is given in Table 4.2. The initial blood glucose level of all the rats before start of the experiment was ranged from 86.25 to 89.50 mg/dl. But the blood glucose level was very much increased on 3rd day of alloxan injection and it was ranged between 368.50 and 396.75mg/dl in both diabetic control and experimental groups. When the experimental duration prolonged, the blood glucose level was positively decreased with respect to the variation in concentrations of sodium alginate treatment. On the final day (45th day) of the experiment, the blood glucose level observed in the lowest concentration of 250 mg/kg sodium alginate treated rats was 145.75 mg/dl, whereas at 500 – 1250 mg/kg concentrations of sodium alginate treated rats, the blood glucose level observed was 138.00, 106.50, 89.25 and 86.25 mg/dl, respectively. On the other hand in the diabetic control group, the blood glucose level
recorded was 252.25mg/dl at the end of the experiment. The Two way ANOVA test conducted for the data on blood glucose level as a function of variation between control and different concentrations of sodium alginate treated groups was statistically more significant (F= 49.75883; P< 0.0001), similarly the variation between different days intervals of experiment was also statistically more significant (F= 19.47787; P< 0.0001) (Table 4.2a). Simultaneously, the statistical one way ANOVA test conducted for the data on blood glucose level on 45th day of experiment as a function of variation between control and different concentrations of sodium alginate treated experimental groups was highly significant (F= 5962.661; P< 0.0001) (Table 4.2b)

4.3.4. Blood hematological parameters

The results on blood hematological parameters of Wistar male albino rats after sodium alginate treatment are given in Table 4.3.

4.3.4.1. RBC level

The RBC level in the normal rat was $3.10 \times 10^6$ mm$^3$, at the beginning of the experiment. But after 3 days of alloxan injection, the RBC level was reduced to $2.60 \times 10^6$ mm$^3$. At the end of 45th day of treatment, the RBC level observed in the diabetic control group was $2.80 \times 10^6$ mm$^3$. Whereas in different concentrations (250 – 1250 mg/kg) of sodium alginate treated groups, the RBC level increased gradually. For instance at 250mg/kg concentration of sodium alginate treated rats, the RBC level observed was $3.10 \times 10^6$ mm$^3$, when the concentration level of sodium alginate increased, the RBC level increased significantly (P< 0.05). Accordingly, the RBC level
observed was 3.30, 3.40, 3.50 and 3.70 x 10^6 mm^3, respectively in 500, 750, 1000 and 1250mg/kg concentrations of sodium alginate treated rats. However, in the normal control group, no change on RBC level was noticed on 45th day of experiment (Table 4.3). The one way ANOVA test conducted for the data on RBC level as a function of variation between control and different concentrations of sodium alginate treated experimental groups was statistically significant (F= 9.938988; P< 0.001) (Table 4.3a).

4.3.4.2. WBC

The WBC level in the normal rat was 8000 cells/cm^2 at the beginning of the experiment, whereas after 3 days of alloxan injection, the WBC level decreased to 5142 cells /cm^2. At the end of the experiment (45th day), the WBC level in the blood sample of diabetic control group was 5300cells/cm^2, whereas in different concentrations (250 – 1250 mg/kg) of sodium alginate treated groups, the WBC level was increased gradually. For instance at 250mg/kg concentration of sodium alginate treated rats, the WBC level observed in the blood sample was 5800 cells/ cm^2. When the concentration of sodium alginate increased, the WBC level was also increased significantly (P< 0.05) and it was observed as 6100, 6600, 7400 and 7900 cells / cm^2, respectively in 500, 750, 1000 and 1250 mg/kg concentrations of sodium alginate treated rats. In normal control group of rats, the WBC level was remaining unchanged till the end of the experiment (Table 4.3). The statistical one way ANOVA test conducted for the data on WBC level as a function of variation between control and different concentrations of sodium alginate treated experimental groups of rats was highly significant (F= 787.8574; P< 0.0001) (Table 4.3a).
4.3.4.3. Haemoglobin (Hb) level

In normal rat, the hemoglobin level recorded was 80% (12.0g), but after 3 days of alloxan injection, the hemoglobin level was drastically reduced to 58% (8.8g). At the end of the experiment (45th day), the hemoglobin level observed in the diabetic control group was 60% (9.0g). But in various concentrations (250 – 1250 mg/kg) of sodium alginate treated groups, the hemoglobin level was increased gradually. For instance at 250mg/kg concentration of sodium alginate treated rats, the hemoglobin level observed was 70% (10.50g). When the concentration level of sodium alginate increased further, the hemoglobin level was also increased significantly (P< 0.05). Accordingly, the hemoglobin level found to be increased as 72% (10.80g) in 500 and 73% (10.90) in 750 mg/kg concentrations of sodium alginate treated rats. But in the highest concentrations of 1000 and 1250 mg/kg sodium alginate treated rats, the hemoglobin level observed was 74% (11.0g) and 75% (11.20g), respectively. However, in the normal control rats, no change on hemoglobin level was observed at the end of 45th day of experiment (Table 4.3). The one way ANOVA test conducted for the data on hemoglobin level as a function of variation between control and different concentrations of sodium alginate treated experimental groups of rats was statistically more significant (F= 119.0323; P< 0.0001) (Table 4.3a).

4.3.4.4. Glycosylated haemoglobin

The glycosylated haemoglobin level observed in the normal rat was 5.78%, whereas after 3 days of alloxan injection, the glycosylated haemoglobin level was
drastically increased to 8.8%. At the end of the experiment (45th day), the glycosylated haemoglobin level recorded in the diabetic control group was 8.12%, but in the experimental groups, it decreased depends on the concentrations of sodium alginate. In experimental groups treated with 250, 500, 750, 1000 and 1250 mg/kg concentrations of sodium alginate, the glycosylated haemoglobin level recorded was gradually decreased to 6.68, 6.28, 6.07, 5.98 and 5.84%, respectively. At the same time in normal control rat, there was no change observed at the end of 45th day of experiment. The one way ANOVA test conducted for the data on glycosylated hemoglobin level as a function of variation between control and different concentrations of sodium alginate treated experimental groups of rats was statistically more significant (F= 26.43107; P< 0.0001) (Table 4.3a)

4.3.5. Blood serum lipid profile

The blood serum lipid profile of Wistar male albino rats after 45 days of sodium alginate treatment is given in Table 4.4.

4.3.5.1. Total blood serum cholesterol

In normal rat, the total cholesterol level recorded in the blood serum sample was 128.0mg%, at the beginning of the experiment, whereas after 3 days of alloxan injection, the cholesterol level increased to 225.0mg%. After 45 days of experiment, the total cholesterol level observed in the blood serum sample of diabetic control group was 205.0mg%. But in different concentrations (250-1250mg/kg) of sodium alginate treated groups, the blood serum cholesterol level decreased gradually, which depends
on the increase in concentrations of sodium alginate. For instance at the lowest concentration of 250mg/kg sodium alginate treated rats, the total cholesterol level recorded in the blood serum sample was 165.0mg%. When the concentration of sodium alginate increased, the total cholesterol level in the blood serum sample was decreased significantly (P< 0.05). It was observed as 162.0, 157.0, 150.0 and 142.0mg%, respectively in 500, 750, 1000 and 1250 mg/kg concentrations of sodium alginate treated rats. However, during 45th day of experiment, no change in the level of blood serum cholesterol was observed in normal control group. The statistical one way ANOVA test revealed that the total cholesterol level in the blood serum samples as a function of variation between control and experimental groups of rats treated with different concentrations of sodium alginate was highly significant (F= 226.7562; P< 0.0001) (Table 4.4a).

4.3.5.2. Blood serum Triglyceride level

The initial triglyceride level observed in the blood serum sample was 112.0mg% in normal rat at the beginning of the experiment, but after 3 days of alloxan injection, the triglyceride level was observed as 182.0mg%. After 45 days of sodium alginate treatment, in alloxan induced diabetic control group, the triglyceride level in the blood serum observed was 170.0mg%. Whereas at different concentrations (250- 1250mg/kg) of sodium alginate treated groups, the blood serum triglyceride level was decreased significantly (P< 0.05), it depends on the increase in concentrations of sodium alginate. Accordingly at the lowest concentration (250mg/kg) of sodium alginate treated rats, the
triglyceride level observed was 155.0mg%. When the concentration of sodium alginate increased, the triglyceride level in the blood serum samples was decreased positively. It was observed as 140.0, 132.0, 126.0, 119.0mg%, respectively in 500, 750, 1000 and 1250 mg/kg concentrations of sodium alginate treated rats. However, no change was observed in normal control rat on 45th day of experiment. The one way ANOVA test revealed that the triglyceride level in the blood serum samples as a function of variation between control and experimental groups of rats treated with different concentrations of sodium alginate was statistically more significant (F= 263.1013; P< 0.0001) (Table 4.4a).

4.3.5.3. Blood serum HDL level

In normal rat, the HDL level observed in the blood serum sample was 28.0mg%, at the beginning of the experiment, after 3 days of alloxan injection, the HDL level drastically increased to 48.0mg%. But after 45 days of sodium alginate treatment, the HDL level recorded in the blood serum sample of diabetic control group was 42.0mg%. However in different concentrations (250- 1250mg/kg) of sodium alginate treated groups, the blood serum HDL level was decreased gradually. For instance at 250mg/kg concentration of sodium alginate treated rats, the HDL level in the blood serum was observed as 39mg%. When the concentration level of sodium alginate increased, the HDL level in the blood serum was decreased significantly (P< 0.05) and it was observed as 37.0, 35.50, 33.50 and 32.0 mg%, respectively in 500, 750, 1000 and 1250 mg/kg concentrations of sodium alginate treated rats. But, no change on HDL level was observed in normal control rats after 45 days of experiment. The statistical one way
ANOVA test revealed that the HDL level in the blood serum samples as a function of variation between control and experimental groups of rats treated with different concentrations of sodium alginate was highly significant \( (F= 64.70635; P< 0.0001) \) (Table 4.4a).

**4.3.5.4. Blood serum LDL level**

The LDL level recorded in the normal control rat was 84.0% at the beginning of the experiment, whereas it extremely increased to 144.0mg% in rats injected with alloxan after 3 days. After 45 days of treatment, in alloxan induced control group, the LDL level in the blood serum sample observed was 135.0mg%. But in different concentrations (250- 1250mg/kg) of sodium alginate treated groups, the blood serum LDL level was decreased significantly \( (P< 0.05) \). For instance at 250mg/kg concentration of sodium alginate treated rats, the LDL level observed was 107.0mg%. When the concentration of sodium alginate increased, the LDL level in the blood serum samples decreased positively to 104.0, 99.0, 93.50 and 90.50mg%, respectively in 500, 750, 1000 and 1250 mg/kg concentrations of sodium alginate treated rats. However in normal control rat, the LDL level remains unchanged (85.0mg%) at the end of the experiment. The statistical one way ANOVA test revealed that the LDL level in the blood serum samples as a function of variation between control and experimental groups of rats treated with different concentrations of sodium alginate was more significant \( (F= 414.6082; P< 0.0001) \) (Table 4.4a).
4.3.5.5. Blood serum VLDL level

In normal rat, VLDL level was recorded as 22.0mg%, but, after 3 days of alloxan injection, the VLDL level increased almost double the value as 42.0mg%. At the end of 45 days of sodium alginate treatment, the VLDL level recorded in the blood serum sample of diabetic control group was 34.0mg%. But in different concentrations (250-1250mg/kg) of sodium alginate treated groups, the blood serum VLDL level was decreased gradually. For instance at 250mg/kg concentration of sodium alginate treated rats, the VLDL level in the blood serum sample observed was 31.50mg%. When the concentration level of sodium alginate increased, the VLDL level in the blood serum was decreased significantly (P< 0.05). Accordingly in 500, 750, 1000 and 1250mg/kg concentrations of sodium alginate treated rats, the VLDL level recorded was 29.50, 27.0, 25.50 and 23.50mg%, respectively. At the same time, no change in the VLDL level of normal control rat was observed till the end of the experiment. The one way ANOVA test revealed that the VLDL level in the blood serum samples as a function of variation between control and experimental groups of rats treated with different concentrations of sodium alginate was statistically more significant (F=88.78378; P<0.0001) (Table 4.4a).

4.3.6. Kidney function test

The results recorded on kidney function tests such as urea, creatinine and uric acid level of Wistar male albino rats after sodium alginate treatment are given in Table 4.5.
4.3.6.1. Urea

The initial urea level observed in the blood serum samples of normal rats was 21.0mg%, but in alloxan induced rats, the urea level recorded in the blood serum sample was 32.0mg% on 3\textsuperscript{rd} day. On the final day of experiment (45\textsuperscript{th} day), the urea level observed in the diabetic control group was 25.50mg%, whereas in different concentrations (250- 1250mg/kg) of sodium alginate treated groups, the blood serum urea level was decreased significantly (P< 0.05). For instance at 250mg/kg concentration of sodium alginate treated rats, the urea level observed was 24.50mg%. When the concentration of sodium alginate increased, the urea level in the blood serum was decreased. It was decreased as 23.0, 22.0, 21.50 and 20.50mg% in 500, 750, 1000 and 1250 mg/kg concentrations of sodium alginate treated rats, respectively. However, there was no change observed in the normal control rat after 45 days of experiment. The one way ANOVA test conducted for the data on urea level in blood serum samples as a function of variation between control and different concentrations of sodium alginate treated experimental groups was statistically significant (F= 18.13818; P< 0.0001) (Table 4.5a).

4.3.6.2. Creatinine

In normal rat, the creatinine level observed in the blood serum sample was 0.76mg%. After 3 days of alloxan injection, the creatinine level increased to 0.94mg%. At the end of 45\textsuperscript{th} day of experiment, the creatinine level observed in the blood serum samples of diabetic control rat was 0.88mg%, but in different concentrations (250-
1250mg/kg) of sodium alginate treated groups, the blood serum creatinine level was decreased gradually. For instance at 250mg/kg concentration of sodium alginate treated rats, the creatinine level in the blood serum sample observed was 0.84mg%. When the concentration level of sodium alginate increased, the creatinine level in the blood serum samples was decreased positively to 0.80, 0.78, 0.76 and 0.73mg%, respectively in 500, 750, 1000 and 1250 mg/kg concentrations of sodium alginate treated rats. But in normal control rat, the creatinine level remains unchanged till the end of the experiment. The statistical one way ANOVA test conducted for the data on creatinine level in the blood serum samples as a function of variation between control and different concentrations of sodium alginate treated experimental rats was highly significant (F= 189.6429; P< 0.0001) (Table 4.5a).

4.3.6.3. Uric acid

The uric acid level recorded in the blood serum sample of normal rats was 3.80mg%, but in alloxan induced rats, the uric acid level in the blood serum samples was increased to 5.90mg% on 3rd day. At the final day (45th day) of experiment, the uric acid level in the blood serum samples of diabetic control group showed 5.40mg%, whereas in different concentrations (250- 1250mg/kg) of sodium alginate treated experimental groups, the blood serum uric acid level was decreased significantly (P< 0.05). For instance at 250mg/kg concentration of sodium alginate treated rats, the uric acid level observed was 5.10mg%. When the concentration of sodium alginate increased, the uric acid level in the blood serum samples was decreased to 4.70, 4.40, 4.10 and 3.90mg%, respectively in 500, 750, 1000 and 1250 mg/kg concentrations of
sodium alginate treated rats. There was no change occurred in normal control group after 45 days of experiment. The one way ANOVA test conducted for the data on uric acid level in blood serum samples as a function of variation between control and experimental rats treated with different concentrations of sodium alginate was statistically significant (F= 18.01217; P< 0.0001) (Table 4.5a).

4.3.7. Enzyme activity in liver and blood samples

The results recorded on enzyme activities in liver tissues and blood serum samples of control and experimental groups of Wistar albino rats are given in Table 4.6.

4.3.7.1. Liver enzymes

4.3.7.1.1. Glycogen phosphorylase

The glycogen phosphorylase activity in the liver tissue samples of normal control rat was 93.12 μmoles of inorganic phosphorus liberated/h/mg protein. Whereas after 3 days of alloxan injection, the glycogen phosphorylase activity in the liver tissue samples of diabetic control rat was drastically increased to 156.12 μmoles of inorganic phosphorus liberated/h/mg protein. At the end of 45th day of experiment, the glycogen phosphorylase activity was decreased with increasing concentrations of sodium alginate treatment. Accordingly it was 123.84, 112.49, 105.48, 99.94 and 96.56 μmoles of inorganic phosphorus liberated/h/mg protein in 250, 500, 750, 1000 and 1250 mg/kg concentrations of sodium alginate treated experimental groups of rats, respectively. At the same time in the diabetic control group, the glycogen phosphorylase activity recorded was 142.26 μmoles of inorganic phosphorus liberated/h/mg protein. But, no
change in glycogen phosphorylase activity was occurred in normal control rat after 45 days of experiment. The one way ANOVA test revealed that the glycogen phosphorylase activity as a function of variation between control and different concentrations of sodium alginate treated experimental groups of rats was statistically more significant (F= 225.5287; P< 0.0001) (Table 4.6a).

4.3.7.1.2. Hexokinase

In normal rats, the hexokinase activity recorded in the liver tissue samples was 0.498 µmoles of glucose phosphorylated/h/mg protein. Whereas after 3 days of alloxan injection, the hexokinase activity was decreased considerably to 0.172 µmoles of glucose phosphorylated/h/mg protein. On 45th day, the hexokinase activity in the liver samples of diabetic control group showed 0.196 µmoles of glucose phosphorylated/h/mg protein, but it was increased in experimental groups with variation in the concentrations of sodium alginate. Accordingly, the hexokinase activity in the liver samples of rats treated with different concentrations (250, 500, 750, 1000 and 1250mg/kg) of sodium alginate displayed 0.284, 0.348, 0.375, 0.422 and 0.457 µmoles of glucose phosphorylated/h/mg protein, respectively. At the same time, there was no change observed in normal control rat. The statistical one way ANOVA test revealed that the hexokinase activity as a function of variation between control and different concentrations of sodium alginate treated experimental groups of rats was more significant (F= 6417.547; P< 0.0001) (Table 4.6a).
4.3.7.1.3. Glucose 6-phosphatase

The glucose 6-phosphatase activity in the liver samples of normal control rats showed 0.196 µmoles of inorganic phosphorus liberated/min/mg protein, but after 3 days of alloxan injection, the glucose 6-phosphatase activity was increased to 0.424 µmoles of inorganic phosphorus liberated/min/mg protein. Whereas at the final day (45th day) of experiment, the glucose 6-phosphatase activity in the liver samples of diabetic control group of rats displayed 0.397 µmoles of inorganic phosphorus liberated/min/mg protein. At the same time in the experimental groups treated with different concentrations of sodium alginate, the glucose 6-phosphatase activity was decreased considerably. Accordingly, it was 0.304, 0.284, 0.248, 0.220 and 0.208 µmoles of inorganic phosphorus liberated/min/mg protein in 250-1250mg/kg concentrations of sodium alginate treated rats, respectively. However, there was no changes occurred in normal control rats after 45 days of experiment. The one way ANOVA test revealed that the glucose 6-phosphatase activity as a function of variation between control and different concentrations of sodium alginate treated experimental groups of rats was statistically more significant (F= 8026.953; P< 0.0001) (Table 4.6a).

4.3.7.1.4. Fructose 1,6-bisphosphatase

In normal control group, the fructose 1, 6-bisphosphatase activity observed in the liver samples of rats was 0.456 µmoles of inorganic phosphorus liberated/h/mg protein. Whereas on 3rd day of alloxan injection, the fructose 1,6-bisphosphatase activity of rats was considerably increased (0.706 µmoles of inorganic phosphorus
liberated/h/mg protein). At the end of 45 days of experiment, the fructose 1,6 bisphophatase activity observed in the liver samples of diabetic control group of rats was 0.654 µmoles of inorganic phosphorus liberated/h/mg protein, but in the experimental groups, it was decreased considerably and reached almost normal level. For instance, in the experimental groups, treated with different concentrations (250, 500, 750, 1000 and 1250 mg/kg) of sodium alginate, the fructose 1,6-bisphosphatase activity observed in the liver samples of rats was 0.576, 0.527, 0.503, 0.488 and 0.472 µmoles of inorganic phosphorus liberated/h/mg protein, respectively. But there was no change recorded in normal control rats. The one way ANOVA test revealed that the fructose 1,6-bisphosphatase activity as a function of variation between control and different concentrations of sodium alginate treated experimental groups of rats was statistically more significant (F= 1194.823; P< 0.0001) (Table 4.6a).

4.3.7.2 Blood serum enzymes

4.3.7.2.1. Aspartate transaminase

In normal control group, the enzyme aspartate transaminase recorded in the blood serum sample was 16.15 IU. But it increased (38.28 IU) after 3 days of alloxan injection. When the duration of experiment increased, the aspartate transaminase activity in the blood serum samples was decreased. At the end of 45th day, the diabetic control group showed the aspartate transaminase activity of 34.25 IU. Whereas it gradually decreased to 26.84, 22.54, 20.21, 17.62 and 16.41 IU in respective concentrations of 250, 500, 750, 1000 and 1250 mg/kg sodium alginate treated rats. However, there was no change recorded in the enzyme activity in the blood serum
samples of normal control rats. The statistical one way ANOVA test revealed that the aspartate transaminase activity as a function of variation between control and different concentrations of sodium alginate treated experimental groups of rats was highly significant (F= 482.3234; P< 0.0001) (Table 4.6a).

4.3.7.2.2. Alanine transaminase

The alanine transaminase activity in the blood serum samples of normal control rats was 12.84 IU, whereas after 3 days of alloxan injection, the alanine activity was increased (30.46 IU) than the normal control. When the duration of experiment increased, the alanine transaminase activity was decreased in different concentrations of sodium alginate treated groups. Accordingly, on 45\textsuperscript{th} day of experiment, the alanine transaminase activity of diabetic control group was 25.64 IU, but it was further decreased to 20.72, 18.28, 16.42, 14.38 and 13.06 IU in 250, 500, 750, 1000 and 1250 mg/kg concentrations of sodium alginate treated groups, respectively. At the same time, there was no change recorded in the alanine transaminase activity in normal control rat till the end of the experiment. The one way ANOVA test revealed that the alanine transaminase activity as a function of variation between control and different concentrations of sodium alginate treated experimental groups of rats was statistically more significant (F= 526.9032; P< 0.0001) (Table 4.6a).

4.3.7.2.3. Alkaline phosphatase

The alkaline phosphatase activity observed in the blood serum samples of normal control rats was 7.54 KA Units. But it increased (22.36 KA Units) after 3 days
of post injection of alloxan. When the duration of experiment increased, the alkaline phosphatase activity was decreased further. On 45<sup>th</sup> day of experiment, the alkaline phosphatase activity of diabetic control group observed was 17.89 KA Units, whereas in the experimental groups treated with different concentrations (250 – 1250 mg/kg) of sodium alginate displayed gradual reduction in alkaline phosphatase activity of 11.48, 9.84, 9.08, 8.31 and 7.96 KA Unit, respectively. In normal control group, no change on alkaline phosphatase activity was recorded. The one way ANOVA test revealed that the alkaline phosphatase activity as a function of variation between control and different concentrations of sodium alginate treated experimental groups of rats was statistically more significant (F= 544.0443; P< 0.0001) (Table 4.6a).

4.3.7.2.4. Lactate dehydrogenase (LDH)

In normal rats, the LDH activity observed in the blood serum samples was 49 IU/L. After injection of alloxan (on 3<sup>rd</sup> day), the LDH activity was drastically increased to 296 IU/L. But at the end of the experimental period (45<sup>th</sup> day), the LDH activity was decreased with increasing concentrations of sodium alginate. Accordingly, in diabetic control group, the LDH activity recorded was 204 IU/L, whereas in different concentrations (250, 500, 750, 1000 and 1250 mg/kg) of sodium alginate treated experimental groups, the LDH activity was positively decreased to 113, 84, 72, 63 and 58 IU/L, respectively. However, there was no change recorded on LDH activity in the blood serum samples of control group of rats. The statistical one way ANOVA test revealed that the LDH activity as a function of variation between control and different
concentrations of sodium alginate treated experimental groups of rats was highly significant (F= 6116.735; P< 0.0001) (Table 4.6a).

4.3.8. Reducing sugar and reducing cholesterol

The results on reducing substances such as sugar and cholesterol recorded in the fecal samples of Wistar male albino rats after 45 days of treatment are given in Table 4.7. In both normal control and diabetic control groups, no reducing sugar level was detected in the fecal samples. At the same time, the reducing cholesterol level detected was 98mg% in normal control rats, whereas it was increased to 105.0 mg% in diabetic control rats. But in the experimental groups, the reducing sugar and cholesterol level were increased with increasing concentrations of sodium alginate. The experimental group with low concentrations (250 and 500mg/kg) of sodium alginate treatment showed 0.5% each of reducing sugar level and 116.0 and 121.0 mg% level of reducing cholesterol, respectively. Whereas in the medium concentration (750mg/kg) of sodium alginate treated rats, 1.0% reducing sugar level and 129.0mg% reducing cholesterol level were observed. But the highest concentrations (1000 and 1250mg/kg) of sodium alginate treated groups displayed 1.5% each of reducing sugar level and 135.0 and 142.0mg% reducing cholesterol level, respectively. The one way ANOVA test conducted for the data on reducing cholesterol level as a function of variation between control and experimental groups of rats treated with different concentrations of sodium alginate was statistically more significant (F= 307.4419; P< 0.0001) (Table 4.7a).
4.3.9. Histopathology study of pancreatic β cells

The histopathology of β cells of pancreatic tissues of control and experimental groups of rats are shown in Plate 4.11 (a to g). In normal control rats, the β cells in the pancreatic tissues showed normal cellular population, but in the alloxan induced diabetic control rats, the β cells of pancreatic tissues were damaged due to increasing level of blood glucose. The β cells in pancreatic tissues of low concentrations (250, 500 and 750 mg/kg) of sodium alginate treated alloxan induced diabetic rats were partially restored. But in the highest concentrations (1000 and 1250 mg/kg) of sodium alginate treated alloxan induced diabetic rats, the β cells of pancreatic tissues were fully regenerated as normal architecture.
4.4. DISCUSSION

Diabetic retinopathy, nephropathy and cardiovascular diseases are among the most common complications of diabetes. Around 85% of all diabetics eventually develop diabetic retinopathy, which is the commonest cause of blindness in the fourth and seventh decades of life (Tewari and Venkatesh, 2004). Diabetes is also one of the leading causes of kidney failure, whereas heart disease accounts for the majority of deaths among people with diabetes in developed countries. To solve this problem, several studies were carried out to treat such disease with many natural plant based products which are having therapeutic effect. Among the products, seaweed based alginates; fucoidans, etc are the excellent sources. In the present study also, the antidiabetic effect of sodium alginate extracted from *S. polycystum* was tested against alloxan induced diabetic rats.

Diabetics usually reduce the body weight of diabetic patients, it depends on the level of sugar in the blood. Even though the medicated patients, the body weight will be varied much. In the present study, the weight of the positive control group of rats was decreased after injection of alloxan, and also in the experimental groups of rats treated with sodium alginate, the body weight was decreased gradually. In accordance with this, Yang *et al.* (2008) have investigated the effects of polysaccharide from *Opuntia monacantha* cladode on body weight of normal and streptozotocin-induced diabetic rats. They reported that the body weight of normal rats increased regularly from 31.1 g to 41.6 g during the term of four weeks, but the diabetic control group
showed a slight decrease in body weight from 31.6 g to 26.9 g. The positive control group received 600 mg/kg body weight of dimethylbiguanide, was determined to maintain constant body weight, when compared to the initial weight (31.2 g) with that of the weight (31.3 g) recorded during fourth week. Comparing data of groups received 100, 200 and 300 mg/kg body weight of polysaccharide of *O*. *monacantha* cladode showed no significant (P> 0.05) difference between them. Xue *et al.* (2009) reported the effect of sulfated *Achyranthes bidentata* polysaccharides (AbP) on body weight of streptozotocin-induced oxidative stress in rats. They pointed out that there was no obvious difference in initial body weight between different groups. After 30 days of experiment, the diabetic control rats gained less body weight than normal control rats. The body weight gains were significantly increased both in sulfated *A*. *bidentata* polysaccharides treated animals and Xiaoke pill-treated ones, when compared with diabetic control rats. But no significant difference was observed in body weight gains between AbP-treated rats and diabetic control. Yu *et al.* (2009) studied the effect of selenium-polysaccharides from the mycelia of *Coprinus comatus* on body weight of alloxan-induced oxidative stress in mice. The alloxan-induced diabetic mice exhibited a significant (P< 0.05) loss of body weight when compared with normal group. The administration of the selenium-polysaccharides and without selenium polysaccharides for 20 days in diabetic mice, there was no significant (P> 0.05) increase in body weight was noticed. Similarly, in the present study also the initial weight of control and experimental groups of rats was ranged between 160.50 and 175.38 g. After 45 days of experiment, the final weight of normal control rats, which received only the saline water
exhibited an increase in weight from 165.80 to 169.40 g. At the same time, in diabetic control and experimental groups of rats, the weight becomes drastically reduced from 4.315 to 9.579%.

Alloxan is widely used to induce experimental diabetes in animals. The mechanism of action in beta-cells of the pancreas is mediated by reactive oxygen species. The action of reactive oxygen species with a simultaneous massive increase in cytosolic calcium concentration causes rapid destruction of beta-cells and resulted in creation of diabetes (Szkudelski, 2001). In the present experiment, the alloxan-induced diabetic rats exhibited a significant increase (P< 0.05) in blood glucose level when compared to normal mice. The administration of sodium alginate (250 – 1250 mg/kg) for 45 days caused a significant (P< 0.05) reduction in blood glucose level, when compared with diabetic control rats. Similar to the present study, Yang et al. (2008) have reported the anti-diabetic effects of polysaccharide from O. monacantha cladode in normal and streptozotocin-induced diabetic rats. They pointed out that the blood glucose level in normal control rats maintained constant during four weeks and was significantly (P< 0.05) lower than those of streptozotocin-induced diabetic rats of the experimental groups. An increasing trend of blood glucose level was found in the control group during four weeks. Polysaccharides from O. monacantha cladode treated group showed significantly (P< 0.05) hypoglycemic effect at fourth week, when compared with the control group and their blood glucose level was not significantly (P> 0.05) varied to the normal control group. Similarly, Chen et al. (2009) studied the anti-diabetic effect of water extract and crude polysaccharides from tuberous root of Liriope
spicata var. prolifera in mice. They suggested that the blood glucose level of diabetic control mice was significantly (P < 0.01) higher when compared with normal control. Water extract (WE) and crude polysaccharides (CPs) of L. spicata were significantly reduced the blood glucose level (66.04% and 65.48%) in diabetic mice after 4 weeks of oral administration than diabetic control mice. Likewise, Yu et al. (2009) reported the protective effect of selenium-polysaccharides from the mycelia of Coprinus comatus against diabetic mice induced with alloxan. They stated that the alloxan-induced diabetic mice exhibited a significant (P < 0.05) increase in fasting blood glucose when compared with normal mice group. The administration of the selenium-polysaccharides for 20 days in diabetic mice caused a significant (P < 0.05) decrease in blood glucose level, when compared with control group. Dewanjee et al. (2009) have reported the antidiabetic potential of swietenine from Swietenia macrophylla seeds in neonatal-streptozotocin induced diabetic rats through oral administration. In this study, swietenine at the dose of 25 and 50mg/kg body weight significantly lowered fasting blood glucose level of diabetic rats in a dose dependant manner.

The assessment of haematological parameters could be used to reveal the deleterious effect of various compounds including plant extracts and polysaccharides on the blood constituents of animals. They are also used to determine the possible alterations in the levels of biomolecules such as enzymes, metabolic products, haematology, normal functioning and histomorphology of the organs (Magalhaes et al., 2008). The alloxan induced diabetic rats have always suffered from anemia, which may be the result of the toxic effect of alloxan. This anemia could be attributed to destruction
of blood cells and reduced rate of its production from the bone marrow to blood. In the present study, the haematological parameters of control and experimental groups of rats were analysed at the beginning (0\textsuperscript{th} day) and at the end of the experiment (45\textsuperscript{th} days). At the beginning of the experiment, the hematological parameters such as RBC (2.60 x 10\textsuperscript{6} mm\textsuperscript{3}), WBC (5142 cells/cm\textsuperscript{2}) and Hb (58.0\%) were decreased in alloxan induced diabetic rats than control group (RBC: 3.10 X 10\textsuperscript{6} mm\textsuperscript{3}; WBC: 8000 cells/cm\textsuperscript{2}; Hb: 80.0\%). At the end of the experiment (45\textsuperscript{th} day), the hematological parameters were increased (RBC: 3.10 to 3.70 x 10\textsuperscript{6} mm\textsuperscript{3}; WBC: 5800 to 7900 cells/cm\textsuperscript{2}; Hb: 70.0 to 75.0\%) with increasing concentrations (250 to 1250mg/kg) of sodium alginate than diabetic control group. Apart from this, the glycosylated hemoglobin level was also higher (8.12\%) in alloxan induced diabetic rats than normal control (5.80\%), whereas it decreased from 6.68 to 5.84\%, respectively in different concentrations (250 to 1250mg/kg) of sodium alginate treated experimental groups. Similarly, Helal \textit{et al.} (2005) have investigated the effect of certain medicinal plants on hematological parameters of diabetic rats, it showed a significant (P< 0.01) reduction in RBC count and Hb concentration in diabetic rats, when compared with control group during the experimental period. Moreover, the groups treated with mixture of \textit{Ferula assa-foetida}, \textit{Boswellia carteriibirdw} and \textit{Comiphora myrrha} showed insignificant (P> 0.05) changes in RBC count and Hb concentration, when compared with control group, while there was a highly significant increase (P< 0.01) of RBC count and Hb concentration, when compared with diabetic group throughout the experiment. \textit{Nigella Sativa} treated group showed highly significant decrease (P<0.01) in RBC count and Hb concentration when
compared with controls group, and no significant changes was recorded in these parameters when compared with diabetic group. On the other hand, the group treated with *Aloe vera* showed significant decrease (P<0.05) in RBC count and Hb concentration, when compared with control group after treatment period, and no significant change was recorded after recovery period. However, there was a significant increase (P<0.05) in these parameters after recovery period when compared with diabetic group. Concerning WBC count, the data showed a significant increase (P<0.05) in diabetic group which was highly significant (P<0.01) after recovery period when compared with control group. The groups treated with mixture of *Nigella Sativa, Aloe Vera, Boswellia Carterii Birdw* and *Comiphora Myrrha* showed insignificant (P>0.05) change in their count when compared with control group during the experiment, while a significant (P<0.05) decrease was recorded after the treatment period and showed highly significant decrease (P<0.01) after recovery period when compared with diabetic group. On the other hand, the group treated with, *Ferula assa-foetida* showed significant increase in its WBC count when compared to control group, while insignificant change was noticed when compared to diabetic group during the study period. Oyedemi *et al.* (2011) reported the effect of aqueous extract of stem bark of *Afzelia africana* on haematological parameters of streptozotocin induced diabetic rats. They observed the significant decrease in the levels of RBC and Hb in the diabetic animals and were drastically increased to near normal level after administration of extract especially at the dose of 200 mg/kg body weight. The level of WBC was slightly
increased after oral administration of the extract at 100 mg/kg, while the dose of 200 mg/kg did not show any effect when compared with the diabetic groups.

The serum lipid characters are usually estimated in diabetes, because elevation might lead to a higher risk for cardiovascular diseases in some cases. Lowering of serum lipid concentration through dietary or drug therapy is associated with a decrease in the risk of cardiovascular diseases (Deedwania and Fonseca, 2005; Weiss and Sumpio, 2006). In the present study, the blood serum lipid profile was estimated at the beginning (0th day) as well as at the end of the experiment (45th days). In the diabetic control group, the total cholesterol (TC) (205.0mg%), triglycerides (TG) (170.0mg%), HDL (42.0mg%), LDL (135.0mg%) and VLDL (34.0mg%) levels in the blood serum were significantly increased than normal control group (TC: 129.0mg%; TG: 114.0mg%; HDL: 28.50mg%; LDL: 85.0mg%; VLDL: 22.50mg%). But blood serum lipid profile was decreased gradually (TC: 165.0 to 142.0mg%; TG: 155.0 to 119.0mg%; HDL: 39.0 to 32.0mg%; LDL: 107.0 to 90.50mg%; VLDL: 31.50 to 23.50mg%) with respect to the variation in the concentrations (250- 1250mg/kg) of sodium alginate treated groups. Similarly, Yang et al. (2008) have investigated the effect of polysaccharides of O. monacantha cladode (POMC) on blood serum lipid profile in streptozotocin induced diabetic rats. They observed the TC, TG and HDL levels of streptozotocin-induced diabetic rats were 1.86, 3.55 and 1.97 mM, respectively. The POMC-treated group of streptozotocin-induced diabetic rats had significantly (P<0.05) lower TC level and higher HDL level than normal rats and diabetic control group. However, no significant (P>0.05) difference was observed
between the TG levels of experimental groups and the control group. Yu et al. (2009) observed the effect of selenium polysaccharides (SPS) from mycelia of *C. comatus* on blood serum lipid profile of alloxan induced diabetic mice. They pointed out that the TC, TG and LDL concentrations in the blood serum were significantly (P<0.05) increased, whereas serum HDL level was significantly (P<0.05) decreased in the alloxan-induced diabetic mice, when compared with the normal mice. In mice treated with polysaccharide for 20 days, the alteration in lipid metabolism was partially attenuated as evidenced by decreasing levels of serum TC and TG and by increasing level of HDL concentration in diabetic mice, and the administration of the SPS (150 mg/kg body weight, organic selenium content: 15.21 µg/g) for 20 days, the alteration in lipid metabolism was partially attenuated as evidenced by decreasing level of blood serum TC, TG and LDL levels and by increased HDL concentration in diabetic mice, when compared with the normal control group. Xue et al. (2009) studied the effect of sulfated *A. bidentata* polysaccharides (SAbP) on blood serum lipid profile of streptozotocin induced diabetic rats. They observed that the total cholesterol, triglyceride and LDL-cholesterol level in blood serum of diabetic control group of rats were significantly (P< 0.01) increased than the normal control rats, but the HDL-cholesterol level was decreased significantly (P< 0.01) than the normal control rats after 30 days of experiment. The SAbP treated group showed significantly decreasing levels of TC, TG, LDL-c, HDL-c, closely to the normal level.

In the present study, the kidney function test such as urea, creatinine and uric acid level were analysed in the blood serum samples at the beginning (0\textsuperscript{th} day) and at
the end of the experiment (45th day). Their results showed significant increase in the level of blood urea (25.50mg%), creatinine (0.88mg%) and uric acid (5.40%), which are the markers of renal dysfunction in the diabetic groups, when compared to control level (Urea: 20.80mg%; Creatinine: 0.76mg%; Uric acid: 3.70mg%). After the treatment of alloxan induced diabetic rats with different concentrations (250 to 1250mg/kg) of *S. polycystum*-sodium alginate, the levels of urea (24.50 to 20.50mg%), creatinine (0.84 to 0.73mg%) and uric acid (5.10 to 3.90mg%) were significantly (*P*< 0.05) decreased than the untreated diabetic group of rats. Similarly, Jarald *et al.* (2008) have investigated the effect of aqueous (polysaccharide) and non polysaccharide extract of *Cynodon dactylon* on kidney function tests in alloxan induced diabetic rats. They observed that the diabetic control rats showed a significant increase in creatinine and urea levels than the normal control rats. But in the aqueous extract (polysaccharide) and non polysaccharide fractions of *C. dactylon* treated groups, the creatinine and urea levels were significantly (*P*< 0.05) decreased than diabetic control group. Likewise Dahech *et al.* (2011) studied the effect of polysaccharide levan isolated from *Bacillus licheniformis* on renal function of alloxan induced diabetic rats. They observed significant increase in creatinine (56%) and urea (60%) contents in the plasma of alloxan-treated diabetic control rats than normal control rats, whereas, the polysaccharide levan significantly (*P*< 0.05) decreased renal dysfunction indices such as urea and creatinine levels in the plasma, when compared with diabetic control rats.

Insulin influences the intracellular utilization of glucose in a number of ways. It also increases hepatic glycolysis by increasing the activity and amount of several key
enzymes. The enzyme hexokinase catalyses the conversion of glucose to glucose-6-phosphatase and plays a central role in the maintenance of glucose homeostasis (Laakso et al., 1995). In the liver, hexokinase is an important regulatory enzyme in the oxidation of glucose (O’Doherty et al., 1999). Being an insulin-dependent enzyme, the hepatic hexokinase activity of diabetic rats is almost entirely inhibited or inactivated due to the absence of insulin (Gupta et al., 1999). This impairment results in a marked reduction in the rate of glucose oxidation via glycolysis, which ultimately leads to hyperglycemia. Glucose-6-phosphatase is a crucial enzyme of glucose homeostasis because it catalyses the ultimate biochemical reactions of both glycogenolysis and gluconeogenesis (Mithievre et al., 1996). Fructose-1,6-bisphosphatase is one of the key enzymes of gluconeogenic pathway. Hepatic glucose production is raised in diabetic state and is associated with the impaired suppression of the gluconeogenic enzyme fructose 1, 6- bisphosphatase. Gluconeogenic enzyme activation is due to the state of insulin impairment because under normal conditions, insulin functions as a suppressor of gluconeogenic enzymes (Pari and Murugan, 2005). Insulin decreases gluconeogenesis by decreasing the activities of key enzymes, such as glucose-6-phosphatase, fructose-1,6-bisphosphatase, phosphoenolpyruvate carboxy kinase, and pyruvate carboxykinase (Murray et al., 2000).

Defects in carbohydrate metabolizing machinery and consistent efforts of the physiological systems to correct the imbalance in carbohydrate metabolism place an overexertion on the endocrine system, which leads to the deterioration of endocrine control. Continuing deterioration of endocrine control exacerbates the metabolic
disturbances by altering carbohydrate-metabolizing enzymes and leads to diabetes (Bailey, 2000). In the present study, the diabetic rats treated with sodium alginate showed significant decrease in the activities of glycogen phosphorylase, glucose-6-phosphatase and fructose-1, 6-bisphosphatase in the liver. However, the hexokinase activity of sodium alginate treated diabetic rats was significantly increased than in diabetic control rats. Sodium alginate may primarily be modulating and regulating the gluconeogenic enzymes through regulation of cAMP or inhibition of gluconeogenesis.

In accordance with these, Malini et al. (2011) have investigated the effect of ellagic acid on glucose metabolism of streptozotocin induced diabetic rats. They reported that the activity of hexokinase was significantly (P<0.05) decreased in liver, whereas the activities of glucose-6-phosphatase and fructose-1, 6-bisphosphatase were significantly (P<0.05) increased in the liver of diabetic rats when compared with normal rats. Oral administration of ellagic acid significantly (P<0.05) increased the activity of hexokinase in liver and decreased the activities of glucose-6-phosphatase and fructose-1, 6-bisphosphatase in liver of streptozotocin induced diabetic rats when compared with diabetic controls. Sudha Rani (2013) have reported the efficacy of aqueous extract of Talinum traiangulare (TT) on carbohydrate metabolism in alloxan induced diabetic rats. They observed that the glycogen phosphorylase activity increased in diabetic rats, whereas glycogen synthase activity decreased. The oral administration of TT leaf extract controlled the above two enzymes activity and maintained normal level of blood glucose. From the literature it is clear that the blood glucose levels are controlled by either utilization or generation of insulin, this process is mediated by glycolysis or
gluconeogenesis or even both. Therefore the activities of glycolytic enzymes and gluconeogenetic enzymes are measured. The activity of hexokinase showed 60% decrease in its activity under diabetes and this activity brought to the normal levels due to the administration of TT treatment. Similarly the enzymes are related to the glucose synthesis like glucose 6 phosphotase and fructose 1, 6 bis phosphotase showed 2 fold increase in the activity under diabetic condition and activities are regulated to normal by the oral administration of TT leaf extract.

Normally, the natural sodium alginate and the three low- molecular weight water- soluble sodium alginates have no effect on the small intestinal absorption of glucose. Natural sodium alginate is hydrolyzed by gastric acid after ingestion and the gelled alginic acid in the stomach. Thus, it appears that the free alginic acid may inhibit glucose absorption from the small intestine. Kato et al. (1991) reported that alginic acid enhanced Na\(^+\) excretion into the faeces in rats fed high salt diets, and they suggested that alginic acid inhibited Na\(^+\) absorption from the small intestine. Glucose absorption from the small intestine occurs through the Na\(^+\) dependent glucose transporter (Peerce and Wright, 1984). Hence, the reduction of blood glucose and insulin elevation by sodium alginate could be due to their inhibition of glucose absorption from the small intestine via their inhibition of Na\(^+\) absorption, and via the gelling of alginic acid in the stomach.

Cholesterol in the diet is absorbed from the intestine and in company with other lipids, incorporated into chylomicrons and very low density lipoprotein (VLDL). Of the cholesterol absorbed, 80-90% is esterified with long chain fatty acids in the intestinal
mucosa. When chylomicron remnants react with the liver, much of their cholesteryl esters are hydrolyzed and the cholesterol taken up by the liver. VLDL formed in the liver transport cholesterol into the plasma. Ultimately, half of the cholesterol eliminated from the body is excreted into the faeces after conversion to bile salts (Kimura et al., 1996). In the present study, the reducing sugar and reducing cholesterol levels were estimated in fecal samples of control and experimental groups of rats. The diabetic control group showed no reducing sugar in the fecal sample, however 105 mg% reducing cholesterol was present. But in the fecal samples of experimental groups of rats, the reducing sugar and cholesterol levels were increased with increasing concentrations of sodium alginate treatment. Kimura et al. (1996) have reported the effects of natural sodium alginate (AG-270) and low-molecular weight alginates (AG-1, AG-5 and AG-10) on cholesterol excretion and glucose tolerance in rats. They found that AG-270 significantly increased cholesterol excretion in the faeces from 24 to 96 h after the oral administration of cholesterol (1 mg/kg), compared to values in the control rats. Low molecular weight alginates AG-5 and AG-10 significantly increased cholesterol excretion in the faeces after 24, 48, 72 and 96 h of cholesterol administration, when compared to the values recorded in the control rats. AG-1 had no effect on cholesterol excretion into the faeces.

In the present study, the histopathological observation of pancreatic β cells of diabetic control group showed degeneration of cells, but in the experimental groups, in particular at the highest concentrations (1000 and 1250 mg/kg) of sodium alginate treated groups, the β cells in the pancreatic tissues were regenerated and observed a
normal architecture of cells. Kulathuran pillai et al. (2012) studied the histopathological observation of pancreatic tissues of alloxan induced diabetic rats treated with ethanolic extract of Cnidoscolus chayamansa. They observed the extensive damage and reduced number of islets of langerhans in pancreas of alloxan induced diabetic rats. But in the experimental groups treated with ethanolic extract of C. chayamansa showed partial restoration of normal cellular population and enlarged size of β-cells with hyperplasia. Hongayo, (2011) have investigated the histological observation of pancreatic tissues of alloxan induced diabetic mice treated with different solvent based extracts of brown alga Cystoseira moniliformis. They found out that an ongoing recovery of the β-cells in the diabetic mice treated with hexane fraction, diethyl ether fraction and aqueous fraction of C. moniliformis extract. Furthermore, it showed similarities with the β-cells in normal non-diabetic mice. Sanadhya et al. (2013) studied the histopathological observation of alloxan induced diabetic rats treated with Anthocephalus indicus fruits. They observed the islets of pancreas of diabetic group rats were degenerated. But in the rats treated with aqueous extract of A. indicus fruits (400 mg/kg bw) islets were normal in arrangement similar to those in normal rats.

The results presented here clearly demonstrate that the orally administered sodium alginate of brown seaweed S. polycystum possesses significant antihyperglycemic action and it could effectively normalise the blood serum lipid profiles (total cholesterol and triglyceride) in alloxan induced diabetic rats. The promising antihyperglycemic efficiency of this sodium alginate demonstrated in this study may open new avenues in the treatment of diabetes and its complications.
4.5. SUMMARY

The present investigation was carried out to study the antidiabetic efficacy of sodium alginate extracted from brown seaweed *S. polycystum* on alloxan induced diabetic rats. The highlights of the study are summarized below.

- Male Wistar albino rats were selected for the present study and were maintained into seven groups viz normal control (without diabetic), diabetic control (Alloxan induced with no treatment) and five experimental groups of alloxan induced with the treatment of 250 – 1250mg/kg concentrations of sodium alginate, respectively. The experiment was conducted for 45 days.

- The weight of the control and experimental groups of rats was measured before and after the experimental period of 45 days. Accordingly, the growth of the diabetic control group was significantly (P< 0.05) decreased (7.015%) after injection of alloxan and also in the experimental groups, the body weight was found to be decreased from 4.31 to 9.57%, respectively with respect to increasing concentrations (250-1250mg/kg) of sodium alginate.

- During the experimental period, the blood glucose level of both control and experimental groups of rats was measured by using digital glucometer. In the diabetic control group, the initial blood glucose level after alloxan injection was 396.75 mg/dl, but it was not reduced much and recorded with 252.25 mg/dl on 45th day. Whereas in the experimental groups, the blood glucose level was...
gradually decreased with respect to the concentration of sodium alginate. Accordingly, on 45th day, the blood glucose level reduced from 145.75 to 86.25 mg/dl in 250 - 1250 mg/dl sodium alginate treated groups, respectively.

- The haematological parameters of control and experimental groups of rats were analysed at the beginning (0th day) and at the end of the experiment (45th day). At the beginning of the experiment, the hematological parameters such as RBC, WBC and Hb were significantly (P< 0.05) decreased in alloxan induced diabetic rats than the normal control rats. Whereas these parameters were significantly (P< 0.05) increased with increasing concentrations of sodium alginate treated experimental groups. Apart from these, the glycosylated hemoglobin level was found to be higher in alloxan induced diabetic rats than normal control rats, but it was significantly (P< 0.05) decreased with respect to the different concentrations of sodium alginate treated experimental groups.

- The blood serum lipid profiles were analyzed after 45 days of experiment. In the diabetic control group of rats, the total cholesterol, triglycerides, HDL, LDL and VLDL levels in the blood serum samples were significantly (P< 0.05) increased than normal control group of rats. But the above blood serum lipid profiles were significantly (P< 0.05) decreased with respect to the increase in concentrations (250-1250mg/kg) of sodium alginate treated rats.

- The kidney function tests were analysed after 45 days of diabetic experiment. The results displayed significant increase in the levels of plasma urea, creatinine
and uric acid in the diabetic control group, which are the markers of renal dysfunction. After the treatment with different concentrations of *S. polycystum*-sodium alginate in alloxan induced diabetic rats, the levels of urea, creatinine and uric acid were significantly (P< 0.05) decreased than the diabetic control rats.

- The diabetic rats treated with sodium alginate showed significant (P< 0.05) decrease in the activities of glycogen phosphorylase, glucose-6- phosphatase and fructose-1, 6-bisphosphatase in the liver samples than the diabetic control rats. However, the hexokinase activity of sodium alginate treated diabetic rats was significantly (P< 0.05) increased than the diabetic control rats.

- The reducing sugar and reducing cholesterol levels were estimated in the fecal samples of control and experimental groups of rats. In diabetic control rats, no reducing sugar and cholesterol were found in the fecal. But in the fecal samples of experimental groups, the reducing sugar and cholesterol levels were significantly (P< 0.05) increased with increasing concentrations of sodium alginate treatment.

- The histopathological observation of pancreatic β cells of diabetic control group showed degeneration of cells, but in the experimental groups, in particular at the highest concentrations of sodium alginate treated groups, the β cells were regenerated and observed a normal architecture of cells.