Sandipan Chatterjee /J. Pharm. Sci. & Res. Vol. 15(11), 2023, 1241-1246

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Evaluation Of Antidiabetic Activity Of Two Marine Algae In Streptozotocin Induced Diabetic Mice

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Abstract:

The study was undertaken to evaluate the marine algae of Sargassum *polycystum* and *Gracilaria edulis* were dried under shade and then powdered, and extracted with 2-2.5 litters of 70% methanol by reflux. Preliminary phytochemical studies were carried out on methanolic extract of SP, and GE. The acute toxicity of Sargassum *polycystum* and *Gracilaria edulis* was performed using mice (18-22g). Diabetes was induced in 16 h fasted Male mice(25-35g) by intraperitoneal injection of 100 mg/kg body weight of streptozotocin. The mice were divided into five groups consisting of six mice each. Goup1 Normal Control, Goup2 Streptozotocin induced mice (65mg/kg i.p.) serves as diabetic control, Goup 3 standard group Insulin injection injected (4U/kg,i.p.), Group 4 Diabetic mice treated with Sargassum *polycystum,* Group 5 Diabetic mice treated *G racilaria edulis.* During study Body weights of mice were taken at end of the treatment using electronic balance. Fasting blood glucose level of rats were taken on before and after the treatment i.e., 0, and 30th day of treatment. At the end of experimental period all the animals were anesthetized. At the end of study, animals in all groups were sacrificed pancreas of each animal was isolated. Biochemical parameters such as total cholesterol, HDL cholesterol, TG, LDL cholesterol, histopathological studies of pancreas performed. The data provided by this study suggest that Sargassum *polycystum* and *Gracilaria edulis* possess potential antidiabetic activity as they significantly lower blood glucose level. These drugs also possess significant antihyperlipidemic activity by lowering serum cholesterol, triglycerides and LDL cholesterol level.

Keywords: Methanolic extract, Sargassum polycystum, Gracillaria edulis, cholesterol, Antiatherogenic, HDL, LDL, TG

1. INTRODUCTION:

The most recent statistics indicate that the global prevalence of diabetes mellitus, estimated as 366 million in 2011, will increase to 522 million by 2030 [1] Diabetes mellitus, a very common and serious endocrine disorder which interferes with the metabolism of carbohydrates, lipids and proteins is caused by the complete or relative insufficiency of insulin secretion and / or resistance to insulin action [2]. The key clinical manifestation of this disorder is chronic hyperglycemia [3] which causes glycation of body protein and so leads to secondary complications affecting the eyes, kidneys, nerves and arteries as well as micro/macro vascular complications and death. Though some non-insulin dependent diabetes mellitus patients can be managed by diet alone, most require oral hypoglycaemic agents and/or insulin therapy. Oral hypoglycemic agents and/or insulin therapy afford relatively effective glycaemic control, but they are not very ideal because of their numerous side effects [4]. Therefore, there is a great need for the development of newer alternative agents that meet the requirement of an ideal antidiabetic compound with little or no adverse side effects. In recent years research interests have shifted to the search for alternative and natural hypoglycemic agents, especially from plant sources [5]. hence, we have chosen to study the activity of two herbal formula on type 1 diabetes mellitus.

2. MATERIALS AND METHODS

2.1 Plant Materials

The marine algae *Sargassum polycystum* and *Gracillaria edulis* was collected during September 2014, from the Mandapam coast (latitude 90 17' Longitude 790 22, E), Gulf of manner. The sample was identified by Scientist in charge, at the Centre for Marine and Fisheries Research Institution (CMFRI), Mandapam Tamil Nadu.

2.2 Preparation of extract

The algae of *Sargassum polycystum* and *Gracilaria edulis* were chopped into small pieces and dried under shade at room temperature for seven days. The dried algae were powdered and passed through the sieve (coarse10/40). The powder was used for the preparation of methanolic extract.

2.3 Extraction and isolation [6]

Dried and powdered algae of *Sargassum polycystum* and *Gracilaria edulis* (each 1.0kg) were extracted with boiling 70% Methanol in a reflux condition. After filtration, the solution was concentrated under a vacuum.

2.4 Experimental Animals

Male Albino mice weighing between 18-35g were procured from NIMHANS animal house and kept in KCP animal house, Bangalore, Karnataka. They were housed, there propylene cage under standard laboratory conditions at room temperature (25° C± 2° C) with 12 h light / dark cycle. The animals were provided with pellet chow and water ad libitum, except during experimentation. The study protocols were duly approved by the Institutional Animal Ethics Committee of Karnataka College of pharmacy,

Bangalore. Studies were performed in accordance with the CPCSEA guidelines.

2.5 Acute oral toxicity study

The acute oral toxicity study was performed using mice (18-22g). according to the Acute Toxic Class method described by OECD guideline 423.[7] The animals were divided into six groups (n=3). One group served as a control and received saline solution orally. The drugs were given in a dose of 300, 500, 2000 and 5000 mg/kg b.w.to each anima orally. The Sargassum polycystum doses were prepared by suspension in gum acacia 1%, and Gracilaria edulis were prepared by dissolving the drug in distilled water. The animals were observed at 0, 30 and 60min, 24, 48 and 72h and 1 week after administration for any kind of behavioural, physical and pharmacological toxic effects, respectively.

2.6 Groups used in the study and Induction of diabetes mellitus [8]

Diabetes was induced in 16 h fasted Male mice(25-35g) by intraperitoneal injection of 100 mg/kg body weight of streptozotocin. Streptozotocin was dissolved in 0.1 M cold sodium citrate buffer (pH 4.5) immediately before use. The mice were then given 5% w/v glucose solution in feeding bottles for the next 24 h in their cages to prevent hypoglycemia. After 72 h, rats with marked hyperglycemic fasting blood glucose> 180 mg/dL were selected and used for the study. All the animals were allowed free access to tap water and pellet diet and maintained at room temperature in polyethylene cages.

The mice were divided into five groups consisting of six mice each.

Goup 1: Administered vehicle serves as Normal control.

Goup 2: Administered Streptozotocin (65mg/kg i.p.) serves as diabetic control

Group 3: Administered Reference standard, Insulin (4U/kg,i.p.)

Group 4: Diabetic mice treated with J1 (500mg/kg,p.o.once daily).

Group 5: Diabetic mice treated with J2 (500mg/kg,p.o.once daily).

Body weights of mice were taken at end of the treatment using electronic balance. Fasting blood glucose level of rats were taken on before and after the treatment i.e., 0, and 30th day of treatment by using one touch ultra glucometer by vein puncture. At the end of experimental period all the animals were anesthetized using anesthetic ether. Blood collected by retro orbital puncture was centrifuged at 2500 rpm for 15 minutes and analyzed for various biochemical parameters.

2.7. Biochemical Evaluation

The biochemical parameters like Glucose, Triglyceride, Total Cholesterol, HDL, LDL, VLDL were estimated as per the standard procedure prescribed by the manufacturer's instruction manual provided in the kit. (Delta Labs kit, Bengaluru, India) using Semi Auto analyser.

2.8. Statistical analysis

Results have been reported as mean value ± SEM. The variation in a set of data has been estimated by performing one-way analysis of variance (Anova). Individual comparisons of group mean values were done using Dunnet's test (Graph pad prism 6.0).

2.9 Histopathological Studies

2.9.1. Pancreas dissection

The animals were euthanized using anesthetic ether and then sacrificed and the pancreas of each animal was isolated and was cut into small pieces, preserved and fixed in 10% formalin for two days. Then the pancreas piece was washed in running water for about 12 hours to remove the formalin and was followed by dehydration with isopropyl alcohol of increasing strength (70%, 80% and 90%) for 12 hours each. Then finally dehydration is done using absolute alcohol with about three changes for 12 hours each. Dehydration was performed to remove all traces of water. Further alcohol was removed by using chloroform and chloroform removed by paraffin infiltration. The clearing was done by using chloroform with two changes for 15 to 20 minutes each. After paraffin infiltration the pancreas pieces were subjected to automatic tissue process in git.

2.9.2. Embedding in paraffin vacuum

Hard paraffin was melted and the hot paraffin was poured into L-shaped blocks. The pancreas pieces were then dropped into the molten paraffin quickly and allow it to cool.

2.9.3. Sectioning:

The blocks were cut using microtome to get sections of thickness of 5. The sections were taken on a micro slide on which egg albumin i.e., sticking substance was applied. The sections were allowed to remain in an oven at 600C for 1 hour. Paraffin melts and egg albumin denatures, thereby fixing tissue to slide.

2.9.4. Staining

Eosin is an acid stain; hence it stains all the cell constituents pink which are basic in nature i.e., cytoplasm. Haematoxylin, a basic stain which stains all the acidic cell components blue i.e.: DNA in the nucleus

3. RESULTS

The LD50 of the extract of *Sargassum polycystum* and *Gracilaria edulis* was found to be 5000mg/kg. So, 1/10th of dose was selected and the experiment was carried out. Effect of methanolic extract of *Sargassum polycystum* and *Gracilaria edulis* (500mg/kg.po/day/30days) on Streptozotocin (100mg/kg.ip/single dose) treated mice on body weight, serum glucose, cholesterol, triglyceride, HDL, LDL, VLDL, after 30 days of treatment.

3.1 Effect on body weight

Administration of Streptozotocin (100mg/kg. ip/single dose) to Swiss albino mice showed significant decrease ($p < 0.001$) in the body weight on 30th day of treatment when compared with normal control. Administration of

Sargassum polycystum and *Gracilaria edulis* (500mg/kg.po/day/30days) and (Insulin 4U/kg.ip/30 days) to the Streptozotocin (100mg/kg. ip/single dose) treated Swiss albino mice showed significant increase (P<0.001) body weight on 30th day of treatment when compared with diabetic control. There was no significant difference between the three treatments.

3.2 Effect on blood glucose

Administration of Streptozotocin (100mg/kg. ip/single dose) to Swiss albino mice showed significant increase (p<0.001) in the blood glucose on 30th day of treatment when compared with normal control. Administration of *Sargassum polycystum* and *Gracilaria edulis* (500mg/kg.po/day/30days) and Insulin (4U/kg.ip/30 days) to the Streptozotocin (65mg/kg. ip/single dose) treated Swiss albino mice showed significant decrease (p<0.01) in the blood glucose on 30th day of treatment when compared with diabetic control. *Sargassum polycystum* and *Gracilaria edulis* gave similar results as compared to the standard Insulin (4U/kg.ip/30 days).

3.3. Effect on serum total cholesterol level

Administration of Streptozotocin (100mg/kg. ip/single dose) to Swiss albino mice showed significant increase (p<0.001) in the serum TC on 30th day of treatment when compared with normal control. Administration of *Sargassum polycystum* and *Gracilaria edulis* and Insulin (4U/kg.ip/30 days) (500mg/kg.po/day/30days) to the Streptozotocin (100mg/kg. ip/single dose) treated Swiss albino mice showed significant decrease (p<0.001) in the serum TC on 30th day of treatment when compared with diabetic control. *Gracilaria edulis* gave a significant decrease as compared standard and *Sargassum polycystum* (P<0.01). However, there was no significant difference between *Gracilaria edulis* and the standard (P>0.05).

3.4. Effect on serum triglyceride level

Administration of Streptozotocin (100mg/kg. ip/single dose) to Swiss albino mice showed significant increase (p<0.001) in the serum TG on 30th day of treatment when compared with normal control. Administration of *Sargassum polycystum* and *Gracilaria edulis* and Insulin (4U/kg.ip/30 days) mg/kg.po/day/30days) to the Streptozotocin (100mg/kg. ip/single dose) treated Swiss albino mice showed significant decrease (p<0.001) in the serum TG on 30th day of treatment when compared with diabetic control. There was no significant difference between *Gracilaria edulis* and the standard (P>0.05).

3.5. Effect on serum LDL level

Administration of Streptozotocin (100mg/kg. ip/single dose) to Swiss albino mice showed significant increase (p<0.001) in the serum LDL level on 30th day of treatment when compared with normal control. Administration of *Sargassum polycystum* and *Gracilaria edulis* (500mg/kg.po/day/30days) and Insulin (4U/kg.ip/30 days) to the Streptozotocin (100mg/kg. ip/single dose) treated Swiss albino mice showed significant decrease (p<0.001) in the serum LDL level on 30th day of treatment when compared with diabetic control.

3.6. Effect on Hepatic enzymes (sGOT and sGPT)

Administration of Streptozotocin (100mg/kg. ip/single dose) to Swiss albino mice showed significant increase (p<0.001) in the serum sGOT and sGPT enzymes level on 30th day of treatment when compared with normal control. Administration of and *Gracilaria edulis* (500mg/kg.po/day/30days) and Insulin (4U/kg.ip/30 days *Sargassum polycystum*) to the Streptozotocin (100mg/kg. ip/single dose) treated Swiss albino mice showed significant decrease (p<0.01) in the serum VLDL level on 30th day of treatment when compared with diabetic control.

Table 1. Effect of *Sargassum polycystum* and *Gracilaria edulis* (500mg/kg.po/day/30days) on Streptozotocin) treated (100mg/kg.ip/single dose mice on body weight, serum glucose, cholesterol, triglyceride, HDL,LDL, VLDL, after 30 days of treatment

Group	Body weight	blood glucose (mg/dl)	TC (mg/dl)	TG (mg/dl)	LDL (mg/dl)	SGOT (UI/L)	SGPT(UI/L)
Normalcontrol	25.86 ±0.31	93.31 ±1.44	74.55 ±2.50	88.61 ±1.27	28.06 ±0.62	159.33 ± 1.53	95.91 ±1.01
Diabeticcontrol	15.88± 1.05***	257.28± 3.29***	153.10± 2.14***	138.95± 1.24***	47.56± 0.68***	293.50 ± 2.59***	266.35± 1.25 ***
STZ 100mg/kg+ *Sargassum polycystum* **500mg/kg**	24.80 ±0.31***	118.25 ±1.97***	114.13 ±2.57***	118.45 ±1.11***	35.98 ±1.39***	187.30 ± 0.95***	128.28 ± 1.64 ***
STZ 100 mg/kg+ *Gracilaria edulis* **500mg/kg**	24.83 ±0.48***	106.75 ±3.39***	125.62 ±2.23***	115.65 ±2.33***	33.13 ±1.45***	134.10 ±1.36***	136.03 ±1.04 ***
STZ 100 mg/kg+ insulin 4U/kg	24.80 ±0.74***	105.20 ±2.96***	111.38 ±3.13***	108.53 ±1.93***	30.56± 0.55***	118.28 ± 2.96***	110.88 ±1.06 ***

3.7 Effect of *Sargassum polycystum (*J1*)* and *Gracilaria edulis (*J2*)* (500mg/kg.po/day/30days) on Streptozotocin (100mg/kg. ip/single dose) treated mice on pancreatic histology after 30 days of treatment.

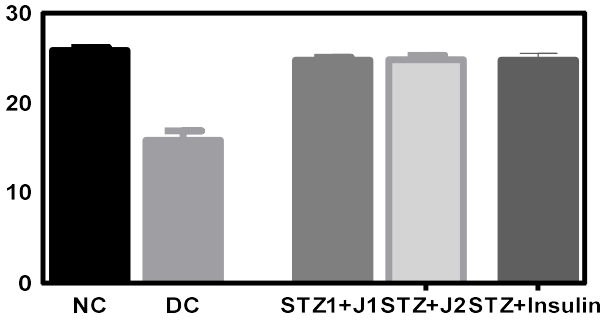


Fig. 1: Effect *Sargassum polycystum (*J1*)* and *Gracilaria edulis (*J2*) on* Streptozotocin (100mg/kg.ip/single dose) treated mice body weight

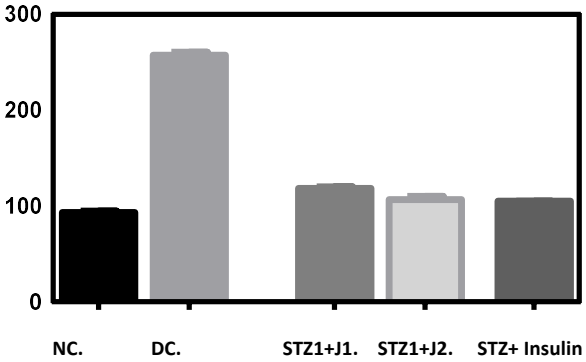


Fig. 2: Effect of *Sargassum polycystum (*J1*)* and *Gracilaria edulis (*J2) (500mg/kg.po/day/30days) on Streptozotocin (100mg/kg.ip/single dose) treatedmice Blood glucose level.

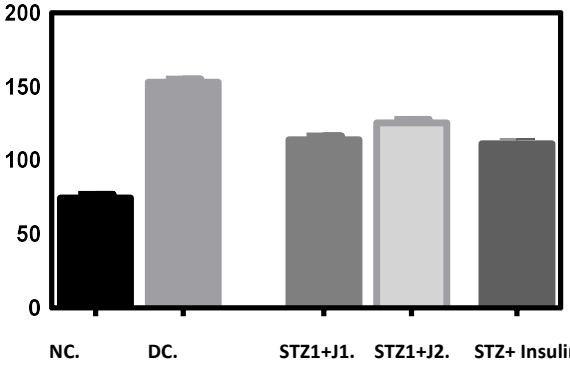


Fig.3: Effect of *Sargassum polycystum (*J1*)* and *Gracilaria edulis (*J2) (500mg/kg.po/day/30days) on Streptozotocin (100mg/kg.ip/single dose) treated mice total cholesterol.

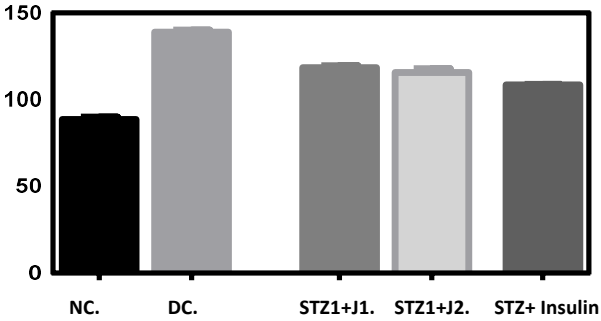


Fig .4: Effect of *Sargassum polycystum (*J1*)* and *Gracilaria edulis (*J2 (500mg/kg.po/day/30days) on streptozotocin (100mg/kg.ip/single dose) treatedmice on triglyceride.

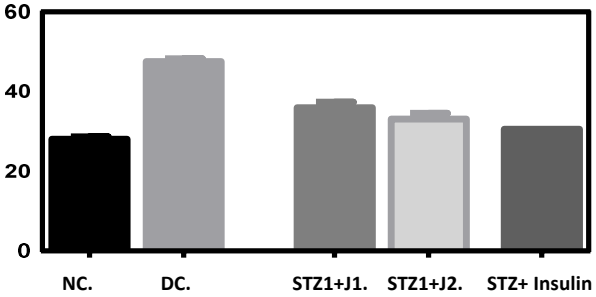


Fig.5: Effect of *Sargassum polycystum (*J1*)* and *Gracilaria edulis (*J2) (500mg/kg.po/day/30days) on Streptozotocin (100mg/kg.ip/single dose) treated mice's serum LDL level.

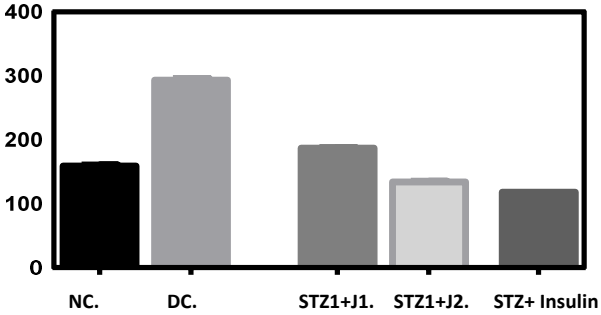


Fig.6: Effect of *Sargassum polycystum (*J1*)* and *Gracilaria edulis (*J2) (500mg/kg.po/day/30days) on Streptozotocin (100mg/kg.ip/single dose) treatedmice's SGOT Enzyme level.

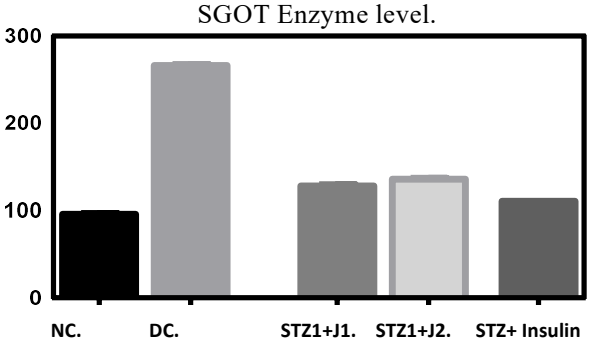
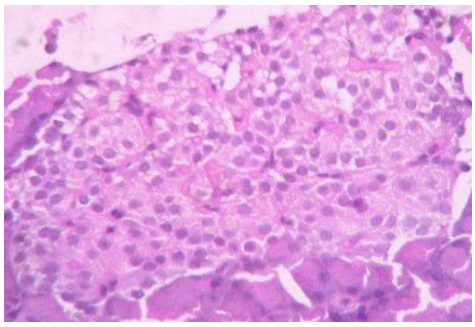


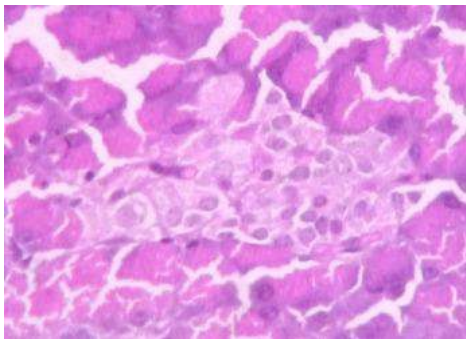
Fig.7: Effect of *Sargassum polycystum (*J1*)* and *Gracilaria edulis (*J2) (500mg/kg.po/day/30days) on Streptozotocin (100mg/kg.ip/single dose) treated mice's SGPT Enzyme level.

3.8 Normal Control (Fig 8)

Pancreatic lobules separated by connective tissue septa refers pancreatic lobules which consist largely of the exocrine acini. Most of the lobules show small, round, light-staining islets of Langerhans. The centre of islet cells consists of aggregates of small β-cells (75%) having basophilic granules, while the periphery comprised of large α-cells (25%) were having eosinophilic granules. Thin-walled capillaries are seen in between these cells.



a) **Fig 8 Section of pancreas of Normal control**



b) **Fig 9 Section of diabetic control**

3.9 Diabetic Control (fig. 9)

Pancreatic lobules separated by connective tissue septa. The number of islets appears reduced in number compared to normal. The centre of the periphery comprises of large α-cells (65%) having eosinophilic granules. This refers degeneration of beta cells islet cells consist of quantitative decrease in β-cells (30%) having basophilic granules.

3.10 STZ+ insulin 4u/kg treated (Fig. 10.)

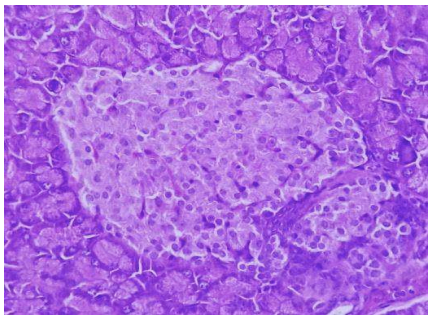
Section studied showed pancreatic lobules separated by thin fibrovascular septa. The centre of islet cells consists, of mild quantitative decrease in β-cells (50%) having basophilic granules, while the periphery comprises of α-cells (55%) having eosinophilic granules. Also seen were few degenerated beta cells.

3.11. STZ+ *Sargassum polycystum* 500mg/kg treated (Fig. 11.)

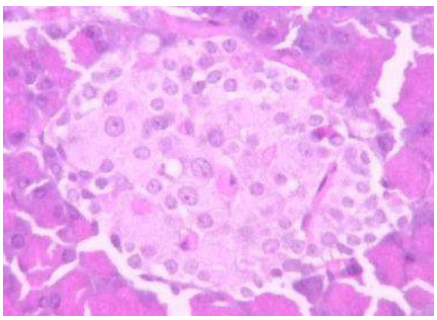
Section studied showed pancreatic lobules separated by connective tissue septa. Most of the lobules showed larger areas of light-staining islets of langerhans. The centerof islet cells consist of quantitative increase in β-cells (65%) having basophilic granules, while the periphery shows slight decrease in α-cells (20%) having eosinophilic granules.

3.12. STZ+ *Gracilaria edulis* 500mg/kg treated (Fig: 12.)

Section studied shows pancreatic lobules separated by fibrovascular septa. The pancreatic lobules consist of intact acinar cells and their intralobular ducts. Most of the lobules show light-staining islets of Langerhans. The centre of islet cells consists of quantitative decrease in Beta-cells compare to high dose 50%) having basophilic granules, while the periphery comprises of decrease in compare to high dose Alpha-cells (23%) having eosinophilic granules.



c) Section of pancreas of Insulin (4u/kg)



d) Section of pancreas of ***Sargassum polycystum*** (500mg)

4. Discussion

STZ is reported to produce free radicals in the body, which specifically cut DNA chains in the pancreatic beta cells, resulting in disorder of the function of the pancreatic beta cells and at a later phase, destruction of the beta cells by necrosis leading to type I diabetes. *Sargassum polycystum* and *Gracilaria edulis* significantly increased blood glucose level in stz treated mice. Diabetes induced by STZ is associated with loss of body weight, this may due to increased muscle wasting due to loss of tissue proteins and fats. *Sargassum polycystum* and *Gracilaria edulis* treated diabetic animals showed marginal increase in body weight as compared to the diabetic control, which may be due to the improvement in glucose uptake, insulin secretion and glycaemic control. Uncontrolled diabetes mellitus, is associated with increase in total cholesterol, triglycerides and LDL cholesterol associated with decrease in HDL cholesterol. Type I diabetes is associated with lower rates of cholesterol synthesis and increased absorption of dietary cholesterol. These individuals are at high risk for the development of cardiovascular disease, and have higher total serum cholesterol levels. In present study, in diabetic control group, there was marked increase in total cholesterol, LDL cholesterol and TG. hyperlipidemia is a known complication of diabetes mellitus and coexists with

hyperglycemia and is characterized by increased level of cholesterol, TG and LDL cholesterol, and all the lipid abnormalities associated with diabetes was significantly normalized by treatment with *Sargassum polycystum* and *Gracilaria edulis*. The histological study of streptozotocin treated mice's pancreas showed significant decrease in beta cell density, whereas *Sargassum polycystum*, *Gracilaria edulis* and insulin treated diabetic mice shows significant increase in beta cell density indicating insulin secretogoge activity. This property may be due to regenerating activity on the beta cells. Liver disease may occur as a result of diabetes mellitus. examples are glycogen seposition, steatosis and non-alcoholic steatohepatitis, fibrosis and cirrhosis. Biliary disease, cholelithiasis and cholecystitis. these conditions are manifested by the increase of hepatic enzymes in serum [9]. In this study treatment of mice with streptozotocin 100mg increased significantly the level of SGOT and SGPT as compared to normal control ($p<0.001$). treatment with *Sargassum polycystum* and *Gracilaria edulis* decreased significantly SGOT and SGPT activities ($p<0.001$ as compared to diabetic control.

5. Conclusion

The data provided by this study suggest that *Sargassum polycystum* and *Gracilaria edulis* possess potential antidiabetic activity as they significantly lower blood glucose level. These drugs also possess significant antihyperlipidemic activity by lowering serum cholesterol, triglycerides and LDL cholesterol level. Further studies are needed to characterize the anti-diabetic activity of these drugs in order to find out the exact mechanism involved in their antidiabetic activity for their further development as new antidiabetic drugs.

Acknowledgements

The authors are grateful to Professor Basavaraj Ramnal, Chairman KCGI, and Director, Dr. K Ramesh, Karnataka College of Pharmacy, Bangalore, for the constant support and facilities to this study

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