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IN VIVO ANTI DIABETIC AND CARBOHYDRATE METABOLIC ENZYMATIC ACTIVITIES BY *SCINAIA BENGALICA*

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Keywords:

Scinaia Bengalica, Red algae, Type 2 anti-diabetic agent, Streptozotocin (STZ), hyperglycemia, Glucose-6-Phosphatase, Fructose-6-Phosphatase

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ABSTRACT

The red algae *Scinaia bengalica (SB)* has not yet been known for its *in vivo* anti-diabetic property and Carbohydrate metabolic enzymes such as glucose-6-phosphatase, fructose-6-phosphatse, Hexokinase, Glucokinase, Lactate Dehydrogenase(LDH). This study was performed to investigate the ability of SB extract as an anti-diabetic agent on streptozotocin induced diabetic rats. The results revealed significant increase in hemoglobin and glycosylated hemoglobin along with the reduction in hyperglycemia. Further, ethanol extracts of SB-treated animals showed that shrinkage of β cells of Langerhans was restored as evidenced by histological studies and the increased activities of carbohydrate metabolic enzymes during diabetics were reduced significantly and comes to normal level by administrating *SB* extract. Thus the SB might serve as a reliable adjuvant for anti-hyperglycemic effects and may be promising for development of phyto medicines for diabetes.

INTRODUCTION

Diabetes mellitus is a group of metabolic diseases in which there are high blood sugar levels over a prolonged period *("Diabetes Fact sheet N°312" WHO. October 2013.)* Symptoms of high blood sugar include frequent urination, increased thirst, and increased hunger. If left untreated, diabetes can cause many complications Serious long-term complications include cardiovascular disease, stroke, chronic kidney failure, foot ulcers, and damage to the eyes[1]. Acute complications include diabetic ketoacidosis and non ketotic hyperosmolar coma[2].

Glucose-6-Phosphatase is the most important key enzyme of glucose homeostasis, and catalysis the terminal step in gluconeogenesis and glycogenolysis[3,4,5]. The enzyme is mainly found in liver and kidney gluconeogenic tissues, their it plays an important role in glucose production[6]. Fuctose-1.6-Di Phosphatase is one of another key enzyme which catalysis the irreversible reaction in gluconeogenesis pathway and it regulates the pathway[7].

In recent years an increasing number of compounds are isolated from marine algae that possess various biological activities[8,9,10,11]. One of the compound is Bromophenols. these compounds are isolated from marine red algae[12], In general, the main activities related to phenolic compounds, sterols and omega fatty acids are antioxidant and antidiabetic activity[13], lowering LDL cholesterol levels[14].

Scinaia Bengalica is a red algae, it is available in Madras Beach Tamilnadu, India[15,16]. In this algae there is no scientific evidence for having antioxidant as well as anti diabetic activities, Generally Red algae has more antioxidant[17] as well as anti diabetic properties[18] based on this, the current study was designed to investigate the in vivo anti-diabetic property on STZ induced albino rats.

MATERIALS AND METHODS

Red Algae Collection

Algal materials were collected from the Madras coastal regions, Tamilnadu, India and obtained fisher by catching method. The collected red algae were washed with tap water to remove salts and other adhering particles. The whole red algae was dried under shade and then powdered with a mechanical grinder to obtain a coarse powder. Equal quantity of powder was passed through 40 mesh sieve and extracted with ethanol in sox let's apparatus to 60'c. The solvent was completely removed by rotary vacuum evaporator. The extract was freeze dried and stored in vacuum desiccators.

Experimental Induction of Diabetes

Diabetes was induced in the animals fasted overnight by a single intra peritoneal (ip) injection of freshly prepared solution of STZ (Sigma, USA) 35 $mgkg^{-1}$ body weight in 0.1M cold citrate buffer pH4.5[19,20,21]. The animals were allowed to drink 5% glucose solution to overcome the drug-induced hyperglycemia[22]. Control rats were injected with citrate buffer alone as a placebo. Animals were considered diabetic if the blood glucose values were 425 mg dL^{-1} on the third day after STZ injection. After a forth night, rats with moderate diabetes having glycosuria (indicated by Benedict's test for urine) and hyperglycemia with blood glucose range of 200– 300 mg dL^{-1} were used for the experiment. Blood was collected from the eyes (venous pool) by sino-ocular puncture. The animal study Ethical Clearance was obtained from VIT University, Vellore, Tamilnadu, India (No: VIT/IAEC/10^{th} / March/14^{th} /No.31. Dated: March31:2015 0)

Drugs And Chemicals

Streptozotocin, Nicotinamide, saline, acetonitrile (HPLC grade), potassium-di-hydrogen orthophosphate, and all substrates, buffers were purchased from Micro labs, Tamilnadu, India. And the rest of chemicals like standard gallic acid (GA), ellagic acid, catechin, and epicatechin were received from Ranbaxy Research Laboratory, Hyderabad, India.

Acute Toxicity Test

The albino wistar rats were divided into six groups of six animals each. A group received saline (10 ml/kg) by gavage and kept as normal control. A single dose of SB algae extract was administered orally to group 2, 3 and 4 at doses of 50, 500, and 5000 mg/kg b.wt., respectively. The extract did not produce any toxic symptoms of mortality up to the dose level of 5000 mg/kg body weight in the treated animals, and hence it was considered safe for further pharmacological screening. The mortality, measured body weight and behavioural screening were recorded daily during 14 days after the extract administration.

Experimental Design

Studies were carried out using Wistar albino male rats (150–200g), obtained from Indian Veterinary Preventive Medicine (IVPM) (Ranipet, Tamilnadu, India). The animals were grouped and housed in polyacrylic cages (38 × 23 × 10 cm) and maintained under standard laboratory conditions (temperature 25 ± 2°C) with dark and light cycle (12/12 h). The animals were fed with standard pellet diet supplied by the Poultry Research Station (Nandhanam, India), and freshwater ad libitum. All the animals were acclimatized to laboratory conditions for 1 week

before commencement of the experiment. Rats were fasted for 24 h prior to the experiment in mesh-bottomed cages to reduce coprophagia but allowed free access to water except for the last hour before the experiment. The animals were divided into six groups as follows after the induction of STZ-induced diabetes. Diabetes was induced in rats two weeks before starting the treatment. Group I animals were considered as control rats. Group II animals were treated as diabetic STZ-induced rats. Group III diabetic-induced animals were fed with 250 mg kg^{-1} of ethanol extract of SB for six weeks. Group IV diabetic-induced animals were fed with 500 mg kg^{-1} of ethanol extract of SB for six weeks. Group V diabetic rats were given glibenclamide orally (0.6 mg kg^{-1}) in distilled water daily for six weeks, Group VI SB 500 mg kg^{-1} only SB extract for six weeks.

Determination of Blood glucose, Glycosylated hemoglobin (HBA1C) and Hemoglobin levels

All the groups of rats were sacrificed by cervical dislocation after six weeks of treatment, blood was collected and processed for estimation of blood glucose[23], glycosylated hemoglobin (HbA1C)[24] and hemoglobin[25] levels.

Determination of Lipid Profile

Plasma samples were used to measure triglycerides (TG) and cholesterol levels. The biochemical estimation such as serum TC[26], TG, LDL, HDL, and very low density lipoprotein (VLDL)[27] were carried out by the standard methods.

Histopathology

The pancreatic tissues were dissected out and washed with ice cold saline immediately. A portion of pancreatic tissue was fixed in 10% neutral formalin fixative solution for histological studies. After fixation, tissues were embedded 1202 in paraffin, solid sections were cut at 5 mm thickness and the sections were stained with hematoxylin and eosin[28].

Determination of activity of carbohydrate metabolic enzymes.

The activity of carbohydrate metabolic enzymes was determined by liver and kidney supernatant fractions of 6 weeks experimental rats by the method of Balginsky[29], Tashima and Yoshimura[30] respectively. About 1-2 mg of protein was used for both the assays. One enzyme unit is defined as the amount of inorganic phosphate, Pi liberated per gram fresh weight per minute at 37^{o}C for both the phosphatases. Phosphatases estimation was by the method of Fiske and Subbarow[31].

RESULTS & DISCUSSION

Statistical Analysis

Results were analyzed for statistical significance using one way ANOVA followed by Dunnett's test using the graph pad statistical software for comparison with control group and STZ-treated group. A $p>0.05$ was considered as significant.

Effect of SB extract on blood glucose, Hemoglobin (hb), and Glycosylated hemoglobin (HBA1C)

Alterations in the blood glucose, Hb and Hb A1C following treatment of diabetic rats with *SB* extract and glibenclamide are given in (Figures 1(a) and (b)**).** The blood glucose levels were significantly increased in STZ diabetic rats as compared to control. Administration of SB and glibenclamide tended to lower the values close to those of control rats. The effect of SB on blood glucose levels in diabetic rats was more evident than glibenclamide. There was no significant alteration in total hemoglobin levels, while the glycosylated hemoglobin (HbA1C) was significantly higher in diabetic rats compared to control (Group II Figure 1(b)). On treatment with *SB* extract or glibenclamide, HbA1C level was lowered significantly, (Group IV or Group V) compared to untreated diabetic rats (Group II). The effect of *SB* was more distinct than glibenclamide in lowering the HbA1C levels.

Effects of SB extract on blood lipid profile (TC, LDL and VLDL)

The lipid profile such as TC (Figure 1(a) LDL, and VLDL (Figure 1(c) levels were significantly increased in diabetic animals (DC), whereas HDL levels were decreased (Figure 1(c)). Diabetic animals at given SB 250mg kg^{-1} showed no change in TC, HDL, LDL, and VLDL levels compared to STZ. On the other hand, the dosage was increased from 250 to 500 mg kg^{-1} body wt., a significant fall in the TC levels was found compared to diabetic animals. When compared with standard drug (Group V) the fall in TC, LDL, and VLDL was dose dependent and highest reduction in cholesterol noted in 500 mg kg^{-1} group compared to STZ animals. The lower HDL in diabetic rats, increased significantly after administration of the ethanol extract of *SB*. The increment was highest at 500 mg kg^{-1}*SB* (Group V).

Effects of SB extract on body weight and carbohydrate metabolic enzymes

A significant decrease in the body weight of diabetic animals was seen increased by administration of *SB* extract (fig 2). The activities of enzymes hexokinase, glucokinase, LDH were found to be decreased in groupie animals (fig 3(e)&(f),3(g)&(h),3(i)&(j)) where as the activities of gluconeogenic enzymes glucocose-6-phosphatase, fructose-1.6-diphosphatase were

significantly increased in diabetic rats (Fig 3 (a) &(c), 3 (b)&(d)). SB extract had significantly increased hexokinase and glucokinase activity of diabetic animals, whereas the level of gluconeogenic enzymes was decreased.

Histopathological examination of pancreatic tissue

Histopathological sections of pancreas of STZ-induced diabetic rats revealed a significant reduction in the size of islets when compared to control. Further, the study revealed the presence of damaged β-cell population to STZ treatment. On the other hand, the studies on the supplementation of SB extract to the diabetic rats revealed restoration of size of the islets along with β -cells repair. This recovery of the β-cells was recorded as dose-dependent. Standard drug, glibenclamide treated rat pancreatic islets showed partial proliferation of β-cells.(Figure 4(i)-(v)). In diabetes the increased blood sugar levels might be due to either insulin resistance of the body cells or decreased secretion of insulin from β-cells manifested in the decreased serum insulin levels[32].In Diabetes mellitus, a partial or total deficiency of insulin secretion causes derangement in carbohydrate metabolism and changes in enzymatic activity of glucokinase, hexokinase, glucose-6-phosphatase, fructose-1,6-diphosphatase, LDH resulting in depletion of liver and muscle glycogen[33]. Excessive hepatic gluconeogenesis and glycogenolysis associated decreased utilization of glucose by tissue is the fundamental mechanism underlying hyperglycemia in the diabetic state[34]. Our results indicate that the activities of hexokinase, the first and the rate limiting enzyme of glycolysis were significantly decreased in untreated diabetic rats. The decreased level observed in SB extract treated diabetic rats may be because of suppression of hepatic gluconeogenesis and glucose output from liver. The reduction in the serum insulin levels in the STZ-treated rats might be attributed to the reduced secretion of the hormone which might be due to the damage of the β-cells of endocrine pancreas. The STZ selectively destroy the pancreatic cells and induce hyperglycemia. The blood glucose level of *SB* extract fed animals was significantly reduced. The highest decrease was recorded in the 500 mg kg^{-1}*SB* and STZ group. In addition, during diabetes the excess glucose present in circulation reacts with hemoglobin to form glycosylated hemoglobin[35,36]. These findings are in agreement with our studies, in which there was no significant changes observed in total hemoglobin but the glycosylated hemoglobin was significantly higher in diabetic rats. Administrations of SB extract restored to normal the total hemoglobin and HbA1C in diabetic rats by reducing the glucose levels. The lipoprotein levels in the STZ-induced diabetic rats revealed significant alterations in

lipoprotein metabolism[37]. The serum TC content increased significantly in diabetic animals. Since insulin exerts a potent inhibitory effect on lipolysis in adipocytes, insulin deficiency was associated with excess lipolysis and increased influx of free fatty acids to the liver[38]. The animal's revealed better proliferation from the STZ-induced damage when compared to control as well as 500mg kg^{-1} treated animal.

This study suggests that the ethanol extracts of *SB* exerted anti hyperglycemic effect as evidenced by decreased glucose levels, decreased serum lipid levels and alteration in the carbohydrate metabolic enzymes. Therefore one can attribute therapeutic value of this ethanol extracts of *SB* to combat the diabetic condition in rats.

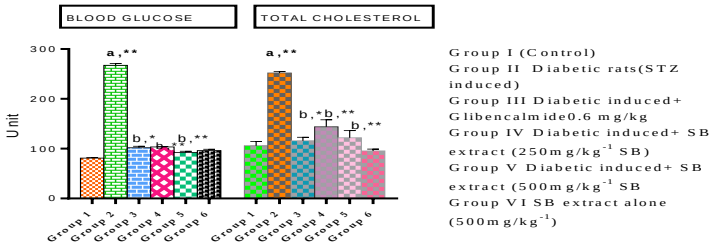

Fig. 1(a) Effect of SB extract on Blood Glucose and Total Cholesterol of STZ induced Diabetic rats.

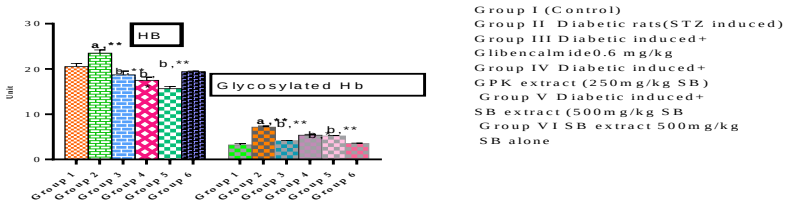

Fig. 1(b) Effect of SB extract on HB and HBA1C of Diabetic induced rats.

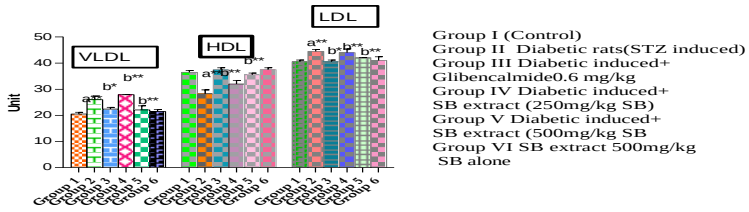

Fig. 1(c) Effect of SB extract on VLDL, HDL, LDL on STZ induced diabetic rats.

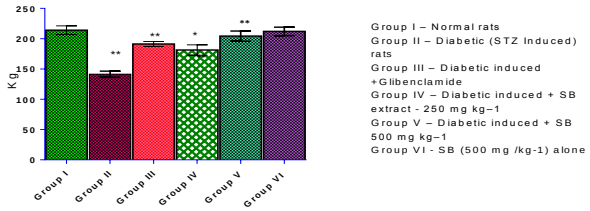

Fig.2 Effect of SB extract on Body weight of STZ induced diabetic rats

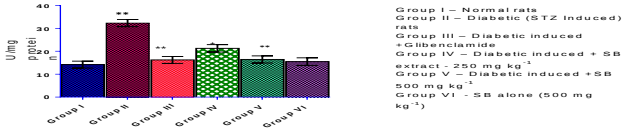

Fig 3(a) Effect of SB extract on Glucose 6 Phosphatase in Liver of STZ induced Diabetic rats.

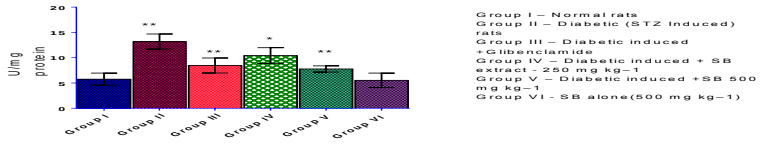

Fig .3(b) Effect of SB extract on the Activity of Fructose-1,6-Diphosphatase in Liver of STZ induced Diabetic rats.

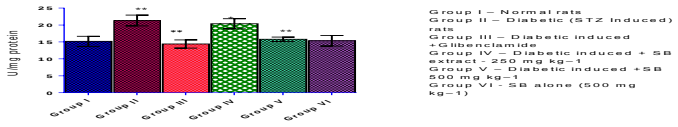

Fig. 3(c) Effect of SB extract on Activity of Glucose-6-Phosphatase in Kidney of STZ induced Diabetic rats.

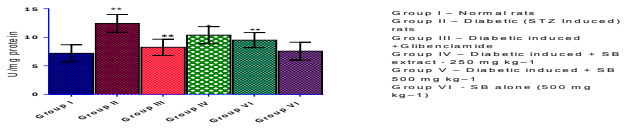

Fig. 3(d) Effect of SB extract on the Activity of Fructose-1, 6-DiPhosphatase in Kidney

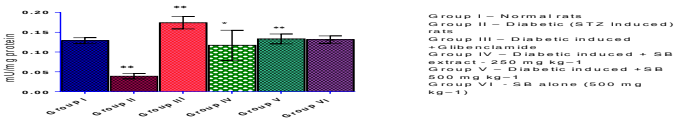

Fig. 3(e) Effect of SB extract on the Activity of Hexokinase in Liver

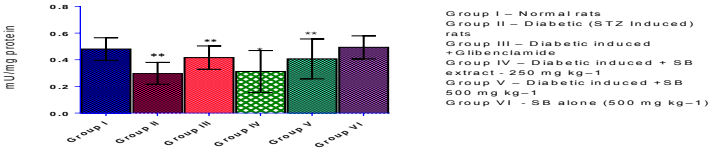

Fig. 3(f) Effect of SB extract on the Activity of Hexokinase in Kidney

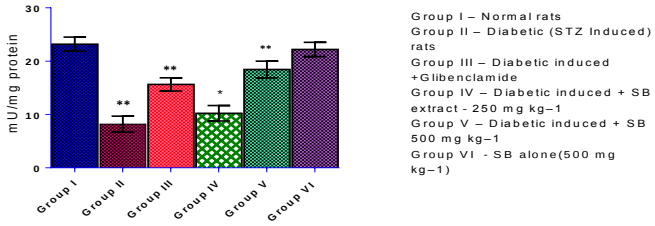

Fig. 3(g) Effect of SB extract on the Activity of Glucokinase in Liver

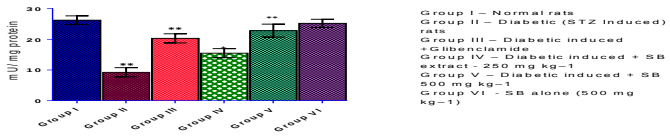

Fig. 3(h) Effect of SB extract on the activity of Glucokinase in Kidney.

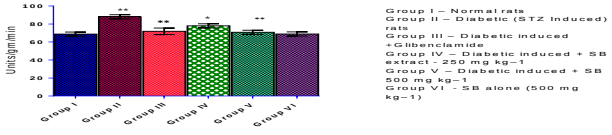

Fig. 3(i) Effect of SB extract on the activity of LDH in Liver

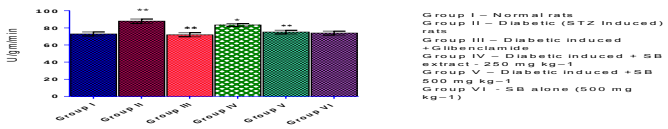

Fig. 3(j) Effect of SB extract on the activity of LDH in Kidney

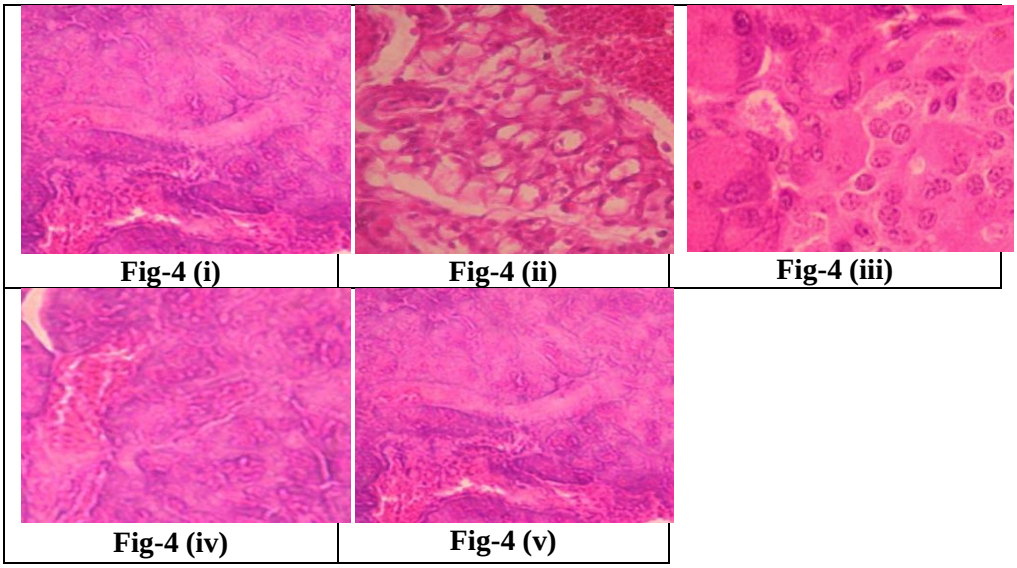

Fig-4 (i) | **Fig-4 (ii)** | **Fig-4 (iii)**

Fig-4 (iv) | **Fig-4 (v)**

Fig-4(i) - The pancreatic islets of Langerhans of normal rats showing alpha cells and beta cells. Fig-4(ii) STZ induced diabetic damaged pancreatic islets showing reduced size and increased damaged beta cells. Fig-4 (iii): *SB* extract (250mg/kg) treated group, pancreatic islets shows partial revealed better restoration, when compared to the STZ induced diabetic control rats. Fig-4(iv) - SB extract (500mg/kg) treated group, pancreatic islets shows partial revealed better restoration, when compared to the STZ induced diabetic and also 250mg/kg treated rats. Fig-4(v) - Diabetic rats treated with Glibenclamide orally (0.6mgkg^{-1}), pancreatic islets show partial proliferation of beta cells.

CONCLUSION

Marine algae derived functional ingredients play a vital role in human health and nutrition. Increasing research on marine algal bioactive compounds raised the demand for novel functional pharmaceuticals. Hence, potent biological compounds from marine algae can be used as functional ingredients to reduce chronic disease in human body.

REFERENCES

1. Kitabchi, AE; Umpierrez, GE; Miles, JM; Fisher, JN (Jul 2009). *"Hyperglycemic crises in adult patients with diabetes."*. Diabetes Care 32 (7): 1335–43. *doi*:*10.2337/dc09-9032*. *PMC 2699725*. *PMID 19564476*
2. David G. Gardner, Dolores (2011). "Chapter 17".Greenspan's basic & clinical endocrinology (9th ed.). New York: McGraw-Hill Medical.*ISBN 0-07-162243-8*.
3. Hers H.G., Van Hoof F & de Barsy T, in The metabolic basis of inherited diseases, edited by C R. Seriver, A.L. Beaudet, R. Charles, W. S. Sly & D. Valle (Mc Graw Hill, New York) 1989.425.
4. Beaudet A L, on Harrison's principle of internal medicine, edited by J D Wilson, E Braunwald, K J Isselbacher, et al, 12th edition (Mc Graw Hill, New York)1991, 1854.
5. Nordie R C& Sukalski K A, in The Enzymes of Biological Membranes, edited by A N Martonusi, 2nd edition, (Penum Press, New York) 1985, 349.
6. Ashmore J & Weber G, Vit Horm, 17(1959)91.
7. Tejwani G A, Pedorsa F O, Pontremoli S & Horeeker B L, Arch. Biochem Biophy, (1976)77.
8. El Gamal AA, Biological importance of marine algae, Saudi Pharm.J.2010:18:1-25.[PMC free article][PubMed]
9. Guven KC, Percot A, Alkaloids in marine algae and . Marine Drugs, 2010:8:269-284.
10. Li K, LiXM, Wang BG. Bromophenols from marine red algae-Rhodomelaceae, Bioorg. Med. Chem.2009:71:28-30[PubMed]
11. Wijesekara I, Pangestuti R, Kim SK, Biological activities and potential health benefits of sulfated polysaccharids derived from marine algae, Carbohydr. Polym. 2011:84:14-21.
12. Li K, Li XM, JiNY, Wang BG. Natural bromophenols from the marine red algae *Polysiphonia urceolata*(Rhodomelaceae): structural elucidation and DPPH radical-scavenging activity. Bioorg. Med. Chem.2007:15:6627-6631.
13. Cardozo, K.H.M., T. Guaratini, M.P. Barros, V.R. Falcao and A.P. Tonon et al., 2007. Metabolites from algae with economic impact. Comparative Biochem. Physiol. C: Toxicol. Pharmacol., 146:60-78.
14. Francavilla M., Colaianna M., Zotti M., Morgese M., Trotta P., Tucci P., Schiavone S., Cuomo V., Trabace L., Extraction, Chraracterization and in vivo neuromodulatory activity of phytosterols from microalgae Dunaliella tertiolecta. Curr.Med.Chem.2012:19:3058-3067. DOI:10.2174/092986712800672021.[PubMed][Cross Ref]
15. Silva, P.C., Basson, P.W. & Moe, R.L.(1996). Catalogue of the benthic marine algae of the Indian Ocean, University Of California Publications in Botany 79: 1-1259.
16. Guiry, M.D. & Guiry, G.M. 2013. AlgaeBase, World-wide electronic publication, National University of Ireland, Galway. http://www.algaebase.org:searched.
17. Lalitha. R, Palani. S., C. Subathradevi., K. Sakthvivel and V. Vahitha-Phytochemical screening by GCMS and *In vitro* antioxidant activity of *GriffithsiaPacifica Kylin: I*nternational journal of Current Microbiology and Applied Sciences. ISSN : 2319-7706 Volume 4 Number 6 2015pp-788-799.
18. Lee, S-H., Han. J-S., Heo, S, J., Hwang, J-Y., & Jeon, Y,-J.(2010). Protective effects of dieckol isolated from Ecklonia cava against high glucose-induced oxidative stress in human umbilical endothelial cells. Toxicology *in Vitro*, 24, 375-381.
19. Bursell SE, Takagi C, Clermont AC, Takagi H, Mori F, Ishii H, King GL. Specific retinal diacylglycerol and protein kinase C beta isoform modulation mimics abnormal retinal hemodynamics in diabetic rats. Invest ophtalmol Vis Sci.1997 Dec; 38(13): 2711-2720.24.
20. Sun Q, Sekar N, Goldwaser I, Genshonov E, Fridkin M, Shechter Y. Vanadate restores glucose 6 phosphate in diabetic rats: a mechanism to enhance glucose metabolism.Am.J physiol Endocrinol metab.2000 Aug;279(2):E403-410.25.
21. Hemalatha S, Wahi A.K, Singh P.N, and Chansouria J.P.N. Hypoglycemic activity ofWithania Coaculants Dunal in streptozotocin Induced diabetic rats. J. Ethnopharmacol 2004; 93:261-264.26.

22. Balasubramaian Ramachandran, Kasiappan Ravi, Vengidusamy Narayanan, Muthusamy Kandaswamy, Sorimuthu Subramaniyan. Protective effect of macrocyclic binuclear oxovanadium complex on oxidative stress in pancreas of sreptozotocin induced diabetic rats. 2004; 149:9-21.27.
23. Sasaki, T., S. Matsy, and A. Sonae. 1972. Effect of acetic acid concentration on the colour reaction in the O-toluidine boric acid method for blood glucose estimation. Rinshokagaku 1: 346–53.
24. Sudhakar, N.S., and T.N. Pattabiraman. 1981. A new colorimetric method for the estimation of glycosylated haemoglobin. Clinica Chemica Acta 109: 267–74.
25. Drabkin, D.S. and Austin, J.H. (1932) Spectophotometric Constants for Common Hemoglobin Derivatives in Human, Dog, and Rabbit Blood. Journal of Biological Chemistry, 98, 719-733.
26. Parekh A.C, Jung D.M. Cholesterol determination with ferric acetate-uranium acetate and sulphuric acid, ferrous sulphate reagents.1970; 42; 1423-1427.28.
27. Burstein R.F and Scholnick V.S, Biochemistry and methodology of lipids.J Lipid Res.1972: 25:375-382.30.
28. Strate T, Mann O, Kleighans H, Rusani S, Schneider C, Yekebas E, Freitag M, Standi T, Bloechle C, Izbicki J.R. Micro circulatory function and tissue damage is improved after the repeutic injection of bovine hemoglobin in server acute rodent pancreatitis. Pancreas. 2005. Apr; 30(3):254-259.
29. Baginsky E S, Foa P P & Zak B, in methods of enzymatic analysis, edited by H U Bergmeyer, 2nd edition (Academic Press, New York)788.
30. Tashima Y & Yoshimura N, J Biochem, 78(1975)1161.
31. Fiske C H & Subbarow Y, J Biol Chem, 66(1925)375.
32. Mohammad Ali E, Razeih Y. (2004). Hypoglycemic effect of Teucrium Polium studies with rat pancreatic islets. J. Ethanopharmacol, 95, 27-30.
33. Hikino H, Koyabshi M, Suzuki Y and Konno C, Mechanism of hypoglycemic activity of aconitan A, a glycan from Aconitum Carmichael roots. Journal of Ethnopharmacology 25:295-304(1989).
34. Latner A Carbohydrate metabolism, abnormalities of post absorptive blood sugar level,in:clinical biochemistry, Philadelphia, WB, Saunders and Co, p.48(1958).
35. Miedema K (2005). "Standardization of HbA1c and Optimal Range of Monitoring". Scandinavian Journal of Clinical and Laboratory Investigation 240: 61-72. *doi:10.1080/00365510500236143*. *PMID 16112961*
36. Peterson KP, Pavlovich JG, Goldstein D, Little R, England J, Peterson CM (1998). What is hemoglobin A1c? An analysis of glycated hemoglobins by electrospray ionization mass spectrometry". Clinical Chemistry (journal) 44(9): 1951-1958. PMID 9732983.
37. Okaraonye C.C and Ikewuchi J.C.(2009). Nutritional and And Antinutritional components of *Pennisetum Purpureum (schumach).* Pakistan Journal of Nutrition 8(1): 32-34 ISSN. 1680-5194.
38. Coppack S.W, Jenson M.D, Milrs J.M. (1994) In vivo regulation of lipolysis in human.J.lipid Res.: 35:177-193.