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Hypoglycemic Potential of *Ascidia sydneiensis* Stimpson, 1855 in Alloxan Induced Diabetic Rats

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Abstract: ***A study of ancient literature indicates that diabetes mellitus was fairly well known as an entity in India and it is one of the major diseases currently affecting millions of people worldwide. Marine organisms have been recognized as rich sources of bioactive compounds with nutraceutical and pharmaceutical potentials. The objective of the present study is to evaluate the hypoglycemic potential of ethanolic extract of Ascidia sydneiensis in alloxan induced diabetic rats with stress on diabetic complications. Oral glucose tolerance, haematological parameters (insulin, glucose, urea, creatinine and glycosylated haemoglobin), serum biochemical, lipid parameters (protein, albumin, globulin, SGPT, SGOT, ALP, TC, TG, HDL-C, LDL-C, VLDL-C, PL) and the level of antioxidant enzymes in plasma (LPO, SOD, CAT, GPX, GSH, GR) were analysed following standard procedures. Administration of the extract at a dose of 100 and 200 mg/kg body weight was compared with control and standard drug Glibenclamide (0.6 mg/kg). The group treated with 200 mg/kg showed potent antidiabetic activity. The ethanol extract of Ascidia sydneiensis elicited significant reductions of blood glucose, urea, creatinine, HbAlc, serum enzymes (SGPT, SGOT and ALP), lipid parameters (TC, TG, LDL-C, VLDL-C and PL), antioxidant enzyme LPO and significant increase of insulin, protein, albumin, globulin, HDL-C, antioxidant enzymes (SOD, CAT, GPX, GSH and GR) compared to control. The serum blood parameters of creatinine and biochemical parameters of protein, albumin and globulin were approaching normal values. Concurrent histological studies of the pancreas showed regeneration on treatment with the extract. From the above results, it is concluded that the ethanolic extract of Ascidia sydneiensis possesses significant hypoglycemic potential against alloxan induced diabetic rats.***

Keywords: *Ascidia sydneiensis*, Antidiabetic activity, Glibenclamide

1. Introduction

Diabetes mellitus is a chronic disease with complex underlying etiologies and the incidence of it is on the rise world wide. Based on the World Health Organisation report [1], the number of diabetic patients is expected to increase from 171 million in year 2000 to 366 million or more by the year 2030 [2]. Drugs of natural origin are considered to be less toxic and free from adverse effects than synthetic ones [3]. The management of diabetics is not without side effects and is a challenge to the medical system. Insulin, oral hypoglycemic agents like sulphonyl ureas and biguanides are still the drugs of choice. As these drugs are to be used throughout life there is diminution of response after long use and side effects [4]. Alternative treatment for diabetes has become increasingly popular during the last several years including medicinal herbs, nutritional supplementation and acupuncture [5]. Ascidians have been screened in a variety of pharmacological bioassays. They are marine invertebrates which ranks second with promising source of drugs [6]. Most of the ascidians are utilized as such as food in various countries and they are known to produce bioactive metabolites which prevent bio-fouling and this can be considered as a kind of autogenic protection [7]. *Ascidia sydneiensis* is a simple ascidian belonging to the family Ascidiidae. Previous studies on this marine species such as taxonomy [8], ecology, distribution, seasonal variation in the occurrence, breeding biology, recruitment and succession in the fouling community, role as bioindicators, food value [9], association with coral reef [10], chemical investigations [11]-[13], antibacterial, antimicrobial activity against human pathogens, *Vibro parahaemolyticus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Alcaligenes* [14],[15] and toxicity [16] are available. There are no reports on the antidiabetic activity of *Ascidia sydneiensis*. Hence the present study focuses on the scientific investigation of antidiabetic activity of the ethanolic extract of *Ascidia sydneiensis*.

2. Materials and Methods

2.1 Animal Material

Samples of *Ascidia sydneiensis* were collected from Tuticorin coast and identified using key to identification of Indian ascidians [17]. A voucher specimen AS 2252 has been deposited in the National Collections of Ascidians in the Museum of the Department of Zoology, A.P.C. Mahalaxmi College for Women, Tuticorin-628002.

2.2 Taxonomic Status

Ascidia sydneiensis is a simple ascidian belonging to the Phylum: Chordata, Subphylum: Urochordata, Class: Ascidiacea, Order: Enterogona, Suborder: Phlebobranchia, Family: Ascidiidae, Genus: *Ascidia*, Species: *sydneiensis*

2.3 Preparation of Extract

For antidiabetic studies, 100 gram powder was extracted with ethanol in Soxhlet apparatus, cooled to room temperature, evaporated in a rotary evaporator under reduced pressure to obtain a brown residue.

2.4 Experimental Animals

180-200 g weight adult male wistar albino rats were obtained from Central Animal House, Annamalai University, Chidambaram, Tamil Nadu, India. Standard environmental conditions of temperature - 24±1°C, 12 h dark-light cycle, free access to drinking water and standard pellet diet were maintained for housing them. Rats were deprived of food except water 16-18 hour prior to the experiments. The rules and regulations of Animal Ethical Committee, Government of India were followed.

a) Induction of Diabetes

Rats were made diabetic by a single intraperitoneal injection of alloxan monohydrate (150 mg/kg) dissolved in sterile normal saline to overnight fasted rats. Since Alloxan is capable of producing fatal hypoglycemia as a result of massive pancreatic insulin release, rats were treated with 20% glucose solution (15 - 20 ml). After 6 hours, the rats were kept for the next 24 hours on 5% glucose solution bottles in their cages to prevent hypoglycaemic shock [18]. After a fortnight rats with moderate diabetes having glycosuria (indicated with Benedict's test for urine) and hyperglycemia with blood glucose range of 190 - 220 mg/100 ml were used for the experiment. All animals were allowed free access to water and pellet diet and maintained at room temperature in plastic cages.

b) Experimental Protocol

Thirty rats were randomly divided into 5 groups of 6 animals each. Non-diabetic control rats and diabetic induced rats were used. The investigation was carried out for 14 days and all the drugs were administered orally using IGC. In these five groups, Group I served as normal and Group II as diabetic control. Both were given normal saline. Group III and IV diabetic rats were given ethanolic extracts of *Ascidia sydneiensis* at doses of 100 and 200 mg/kg bw. Group V was administered with the standard drug glibenclamide (0.6 mg/kg). At the end of experiment rats were subjected to light ether anaesthesia. Blood samples were collected from abdominal aorta and centrifuged at 3000rpm for fifteen minutes at 4°C for separating the serum. The level of glucose was assessed using the frozen serum kept at -20°C. The drug treatment was given to the animals and was fasted for 12 hour before estimating the blood glucose level.

c) Oral Glucose Tolerance

Blood samples were collected just prior to glucose administration taken as zero hour value and after one, two and three hours of glucose loading and their levels were measured by using a glucose oxidase-peroxidase reactive strips and a Glucometer.

d) Estimation of Haematological Parameters

Insulin, glucose, urea, creatinine and glycosylated haemoglobin (HbAlC) were estimated by the procedures [19]-[23].

e) Estimation of Serum Biochemical Parameters

Protein, albumin, globulin, serum glutamate pyruvate transaminase (SGPT), serum glutamate oxaloacetate transaminase (SGOT) and serum alkaline phosphatase (ALP) were measured spectrophotometrically by following methods [24]-[26].

f) Estimation of Lipid Parameters

Serum total cholesterol, triglycerides, high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), very low density lipoprotein cholesterol (VLDL-C) and phospho lipids (PL) were analyzed by standard procedures [27]-[31].

g) Estimation of Antioxidant Enzymes

Lipid peroxide (LPO), super oxide dismutase (SOD), catalase (CAT), glutathione peroxidise (GPX), reduced glutathione (GSH) and glutathione reductase (GR) were determined by standard methods [32]-[37].

2.5 Histopatology of Pancreas

The entire pancreas was removed immediately after sacrificing the animal and rinsed in ice-cold saline. A portion of pancreatic tissue was fixed in 10% neutral formalin for histological studies. The tissues were embedded in parafffin, solid sections were cut at 5 µ thickness and the sections were stained with haematoxylin and eosin [38].

2.6 Statistical Analysis

Values are presented as mean ± S.E.M and statistically evaluated by one-way analysis of variance (ANOVA) followed by student's t - test to identify the differences between *diabetic control and extract treated groups and ^{a}Standard drug and extract treated groups.

3. Results and Discussion

Diabetes mellitus is one of the most common chronic disease associated with hyperglycemia, hyperlipidemia and co-morbidites such as obesity, hypertension. Management of diabetes is still a challenge to the medicinal systems. Though, various types of oral anti-hyperglycemic agents are available in addition to insulin for treatment, these agents are having many side effects [39]. Present study was conceived with a view to provide scientific and pharmacological evidences for hypoglycemic potential of ethanolic extract of *Ascidia sydneiensis* on male wistar rats with stress on diabetic complications. Pancreas is the primary organ involved in sensing the organism's dietary and energetic states via glucose concentration in the blood and in response to elevated blood glucose, insulin is secreted. Alloxan is one of the usual substances used for the induction of diabetes mellitus. It causes a massive reduction in insulin release by the destruction of β-cells of the islets of langherhans, thereby inducing hyperglycemia. Insulin deficiency leads to various metabolic alterations in the animals viz increased levels of cholesterol, alkaline phosphate and transaminases [40],[41].

Table 1. represents the effect of ethanol extract of *Ascidia sydneiensis* on oral glucose tolerance at different time points. 60 minutes after glucose administration, the blood glucose

level increased rapidly from the fasting value and subsequently showed a moderate decrease only after 180 min in diabetic control. Administration of 100 and 200 mg/kg body weight significantly decreased the blood glucose level in a dose dependent manner from 60 minutes onwards suggesting that it has hypoglycemic properties. At the end of the experimental duration, the level of glucose in the group administered with highest dose of extract was less than that of the standard drug treated group.

The effect of the extract on blood parameters is illustrated in table 2. Insulin level was significantly increased in group III and IV treated with extract (10.41±0.36, 14.13±0.36) compared to group II (7.36±0.12). A dose related decrease in the level of glucose, urea, creatinine and glycosylated haemoglobin was noted in groups treated with 100 and 200 mg/kg compared with diabetic control. All the blood parameters were brought back to that of normal. Treatment with *M. malabathricum* leaf significantly reduced blood glucose level in diabetic rats which representing reversal of insulin resistance or increasing insulin secretion possibly by regeneration of damaged pancreatic β-cells [42]. The blood urea and creatinine level significantly decreased which indicates non-toxic effect of the extract on the kidney. HbA1c is considered as a diagnostic marker and helps to estimate the reaction between excess glucose in blood and free amino groups of globulin indicated by protein glycation [43]. The rate of glycation is proportional to the concentration of blood glucose. In the present study administration of the extract showed a significant decrease in HbAlc that could be due to an improvement in glycemic status. Glycosylated haemoglobin increases over a long period of time in diabetes.

The protein, albumin and globulin level were reduced after the induction of diabetes and treatment with the extract increased the levels considerably towards normal (Table 3). This increase may be presumed due to gluconeogenesis during diabetes [44]. It may also be through increase in the insulin mediated aminoacid uptake, enhancement of protein synthesis and/or inhibition of protein degradation [45]. The level of SGPT, SGOT and ALP increased in diabetic control and on treatment, the serum enzymes were restored to their respective normal level indicating non-toxic action of the extract on liver, normal secretion of insulin and hypoglycemic effect. The SGPT and SGOT level increases as a result of metabolic changes in the liver, such as administration of toxin, cirrhosis, hepatitis and cancer [46]. The elevated levels of SGPT and SGOT in alloxan induced diabetic rats may be due to leaking out of enzymes from the tissues and migrating into the circulation by the adverse effect of alloxan [47]. The effect of the extract on the recovery of hepatic enzyme activity in serum was very similar to that of glibenclamide.

Table 4 indicates the serum lipid parameters. The present study showed a dose dependent decrease in the levels of TC, TG, LDL-C, VLDL-C and PL while HDL-C significantly increased when compared to that of diabetic control. The different parameters studied except HDL-C were higher in group II when compared to that of normal control whereas the values were more close to the group treated with glibenclamide. Lipids play a vital role in the pathogenesis of diabetes mellitus. The levels of serum lipids are usually elevated in diabetes mellitus and such an elevation represents a risk factor for coronary heart diseases. The abnormal high concentration of serum lipids in the diabetic subjects is due, mainly to the increase in the mobilization of free fatty acids from the peripheral fat depots. Acute insulin deficiency initially causes an increase in free fatty acid mobilization from adipose tissue. The most common lipid abnormalities in diabetes are hypertriglyceridemia and hypercholesterolemia [48]-[50]. During diabetes, enhanced activity of the enzyme, increases lipolysis and releases more fatty acids into the circulation promoting the β-oxidation of fatty acids, producing more acetyl Co-A and cholesterol [51]. In normal condition, insulin increases receptor-mediator removal of LDL-C and decreased activity of insulin, during diabetes causes hypercholesterolemia. Significant lowering of total cholesterol and rise in HDL-C is a very desirable biochemical state for prevention of atherosclerosis and ischaemic conditions [52]. Similar observation of antidiabetic activity is reported on treatment with the ethanolic extract of simple ascidian *Microcosmus exasperatus* [53].

The level of antioxidant enzymes in plasma is shown in Table 5. Lipid peroxide level showed a decrease whereas other enzymes like SOD, CAT, GPX, GSH and GR increased in group III and IV in a dose dependent manner. A decrease in the concentration of total antioxidant enzymes in the diabetic control rats may be due to their utilization for destruction of free radical species. The activation of GR plays an important role in elevating the concentration of GSH, which maintains the oxido-redox status in the organism [54]. The brain, which is very vulnerable to free radical damage, has seven times more GPX activity than CAT [55]. SOD, CAT and GPX are enzymes that destroy the peroxides and play a significant role in providing antioxidant defenses to an organism. GPX and CAT are involved in the elimination of H_2O_2. SOD acts to dismutate superoxide radical to H_2O_2, which is then acted upon by GPX. The functions of all three enzymes are interconnected and a lowering of their activities results in the accumulation of lipid peroxides and increased oxidative stress in diabetic rats [56]. Lipid peroxide mediated tissue damage has been observed in the development of both type I and II diabetes. It has been observed that insulin secretion is closely associated with lipoxygenase-derived peroxides [57]. The extract significantly lowered the elevated level of LPO suggesting that it might prevent oxidative stress and provide protection to vital tissue of liver, kidney and heart indicating antioxidant activities [58]. However, further studies would be essentially required to elucidate the exact mechanism of hypoglycemic activity of the ethanolic extract of *Ascidia sydneiensis* and to establish its efficacy and safety for further clinical use in diabetic patients.

The histopathological changes observed in the arrangement of cells of the pancreas are given in plate 1. Normal control showed normal islets. The acinar cells which stained strongly are arranged in lobules with prominent nuclei and the islet cells are seen embedded within the acinar cells surrounded by a fine capsule. In diabetic control, the acinar cells around the islets though seem to be in normal proportion does not look classical. The islets are largely occupied by a uniform eosinophilic material and few atrophic cells. Eosinophilic materials also surround the blood vessel. The group treated with 100 mg/kg bw of *Ascidia sydneiensis* showed normal acinar cells but the islets were with heavy lymphocytic

infiltration in and around it (insulinitis). Some normal islet cells are also present. The acinar cells seen to be normal in 200 mg/kg bw extract treated group with a large proportion of islet cells, though smaller in volume compared to control. There is very scanty inflammatory cell infiltration and no eosinophilic deposits indicating better restoration of β- cells in comparison with low dose treated group. Standard drug glibenclamide administered groups exhibited islet cells resembling normal. In the current investigation, there was a reduction in the number of β-cell expansion of pancreas in alloxan induced diabetic rats which was again normalized in groups treated with ethanol extract. So it can be finalized that the active principles in *Ascidia sydneiensis* may be responsible in repairing the injury caused to the β-cells of the islet in pancreas and initiate hypoglycemic effect.

Table 1: Effect of *Ascidia sydneiensis* on oral glucose tolerance at different time points

Group/ Dose mg/kg	*Blood Glucose levels (mg/dl)*			
	0 hour	*1st hour*	*2nd hour*	*3rd hour*
I-Normal control	69.84±2.56	132.53±8.13	108.36±4.56	78.48±4.64
II-Diabetic control	198.65±8.36	247.45±9.23	238.84±7.38	229.38±9.34
III-100 mg/kg bw	201.46±7.34	164.34±6.53*	123.56±7.49**	104.44±8.45***
IV-200 mg/kg bw	193.49±6.38	138.68±4.86**	118.34±5.38***	93.28±4.58***
V-Glibenclamide 0.6 mg /kg bw	219.48±11.35	131.48±9.34	121.46±9.37	97.36±6.87

Data represented as mean ± SEM, (N=6). Compared with initial blood glucose level (0 hr) in the respective group *P<0.05, **P<0.01; ***P<0.001.

Table 2: Effect of *Ascidia sydneiensis* on haematological parameters

Group/ Dose	*Insulin (MIu/ml)*	*Glucose (mg/dl)*	*Urea (mg/dl)*	*Creatinine (mg/dl)*	*HbA1c (%)*
I-Normal control	13.18±0.16	68.34±1.65	13.56±0.24	0.73±0.09	4.36±0.18
II-Diabetic control	7.36±0.12	248.65±7.54	30.84±0.18	1.98±0.14	12.58±1.84
III-100 mg/kg bw	10.41±0.36^{**aa}	104.52±2.16^{*a}	14.84±0.31^{**aa}	1.13±0.11^{***aaa}	6.39±0.24^{**aa}
IV-200 mg/kg bw	14.13±0.36^{***aaa}	94.56±0.84^{**aa}	12.26±0.18^{***aaa}	0.79±0.06^{***aaa}	5.08±0.23^{***aaa}
V-Glibenclamide 0.6 mg /kg bw	13.94±0.36	98.63±2.08	11.56±0.18	0.78±0.07	4.98±0.18

Data represented as mean ± SEM, (N=6). Significance between *Diabetic control and extract treated group. *P < 0.05, **P < 0.01, ***P < 0.001, ^{a}Standard drug and extract treated ^{a}P < 0.05; ^{aa}P < 0.01; ^{aaa}P < 0.001.

Table 3: Effect of *Ascidia sydneiensis* on serum biochemical parameters

Group/ Dose	*Protein (g/dl)*	*Albumin (g/dl)*	*Globulin (g/dl)*	*SGPT (u/l)*	*SGOT (u/l)*
I-Normal control	8.26±0.13	4.67±0.26	3.59±0.45	12.67±0.13	15.92±0.43
II-Diabeticcontrol	6.58±0.02	3.84±0.07	2.74±0.37	32.16±1.32	36.84±1.56
III-100 mg/kg bw	8.04±0.19^{***aaa}	4.34±0.2^{***aaa}	3.70±0.2^{***aaa}	18.46±0.56^{**aa}	16.28±0.19^{*a}
IV-200 mg/kg bw	8.51±0.84^{***aaa}	4.93±0.27^{***aaa}	3.58±0.78^{***aaa}	15.81±0.18^{**aa}	13.18±0.2^{***aaa}
V-Glibenclamide 0.6 mg/kg bw	8.26±0.23	4.81±0.11	3.45±0.29	17.36±0.24	14.82±0.18

Data represented as mean ± SEM, (N=6). Significance between *Diabetic control and extract treated group. *P < 0.05, **P < 0.01, ***P < 0.001, ^{a}Standard drug and extract treated ^{a}P < 0.05; ^{aa}P < 0.01; ^{aaa}P < 0.001.

Table 4: Effect of *Ascidia sydneiensis* on lipid parameters

Group/ Dose	*TC (mg/dl)*	*TG (mg/dl)*	*HDL-C (mg/dl)*	*LDL-C (mg/dl)*	*VLDL-C (mg/dl)*	*PL (mg/dl)*
I-Normal control	132.65±2.11	104.31±0.98	49.16±0.92	62.63±1.45	20.86±1.31	186.05±4.98
II-Diabetic control	231.56±1.84	141.18±1.36	26.84±0.21	90.38±5.38	28.23±1.64	274.08±6.23
III-100 mg/kg bw	173.16±1.26^{**aa}	121.65±0.98^{**aa}	39.31±0.84^{**aa}	109.52±4.37^{*a}	24.33±1.33^{***aaa}	222.11±5.92^{*a}
IV-200 mg/kg bw	138.16±0.91^{***aaa}	120.30±1.36^{**aa}	42.88±0.14^{**aa}	81.22±2.18^{**aa}	24.06±1.48^{***aaa}	199.86±4.27^{**aa}
V-Glibenclamide 0.6 mg /kg bw	141.31±1.78	118.16±0.36	46.31±0.89	86.27±1.78	23.73±1.68	207.11±5.11

Data represented as mean ± SEM, (N=6). Significance between *Diabetic control and extract treated group. *P < 0.05, **P < 0.01, ***P < 0.001, ^{a}Standard drug and extract treated ^{a}P < 0.05; ^{aa}P < 0.01; ^{aaa}P < 0.001.

Table 5: Effect of *Ascidia sydneiensis* on antioxidant enzymes

Group/ Dose	*LPO (mmol/ml)*	*SOD (u/gm Hb)*	*CAT (u/gm Hb)*	*GPX (U/L)*	*GSH (mmol/ml)*	*GR (U/L)*
I-Normal control	1.78±0.051	786.38±11.95	82.84±1.36	531.56±12.16	52.65±1.18	26.92±1.36
II-Diabetic control	5.84±0.011	443.86±9.31	34.54±0.98	354.65±9.27	26.91±0.8	16.24±1.62
III-100 mg/kg bw	2.92±0.016^{***aaa}	681.56±8.12^{**aa}	63.91±0.27^{**aa}	509.36±12.45^{**aa}	39.54±0.78^{*a}	18.16±0.12^{*a}
IV-200 mg/kg bw	2.52±0.68^{***aaa}	739.56±11.56^{**aa}	82.46±1.18^{***aaa}	529.81±1.56^{***aaa}	59.22±1.16^{**aa}	20.89±0.54^{**aa}
V-Glibenclamide 0.6 mg /kg bw	2.04±0.051	741.84±12.67	80.11±1.35	508.16±8.14	51.26±1.28	21.56±0.24

Data represented as mean ± SEM, (N=6). Significance between *Diabetic control and extract treated group. *P < 0.05, **P < 0.01, ***P < 0.001 ^{a}Standard drug and extract treated ^{a}P < 0.05; ^{aa}P < 0.01; ^{aaa}P < 0.001.

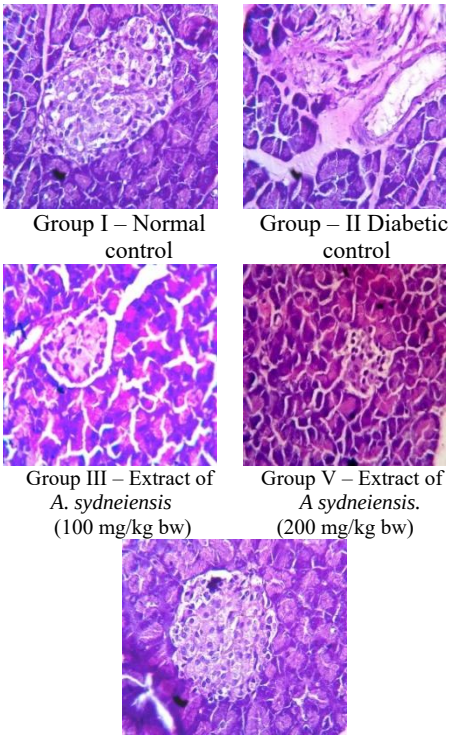

Plate1: Photomicrograph showing histopathological changes in the pancreas

4. Conclusion

The results revealed that the ethanolic extract of *Ascidia sydneiensis* possess significant antihyperglycemic activity as well as improving hyperlipidemia and other metabolic aberrations in alloxan-induced diabetic rats. It has the potential to impart therapeutic effects in diabetes and needs long term studies on its extracts and isolated compounds. Further pharmacological and biochemical investigations are underway to find out the active constituents responsible for hypoglycemic activity and to elucidate its mechanism of action.

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