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Antidiabetic Activity of the Ethanol Extract of Simple Ascidian, *Microcosmus exasperatus* Heller, 1878

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ABSTRACT

Ascidians are an interesting group of marine sedentary organisms. They are known to contain a variety of biologically active compounds with pharmacological properties. The present study aims at analysing the antidiabetic activity of the marine simple ascidian, *Microcosmus exasperatus*. Alloxan induced diabetic albino rats were administered with 100 and 200 mg/kg body weight of the ethanol extract daily for 14 days. Blood glucose, insulin level, urea, creatinine, glycosylated haemoglobin, protein, albumin, globulin, Serum Glutamate Pyruvate Transaminase (SGPT), Serum Glutamate Oxaloacetate Transaminase (SGOT), Alkaline Phosphatase (ALP), Total Cholesterol (TC), Triglycerides (TG), Low Density Lipoprotein Cholesterol (LDL – C), Very Low Density Lipoprotein (VLDL), High Density Lipoprotein (HDL), Phospholipid (PL) and Lipid Peroxide (LP), Super Oxide Dismutase (SOD), Catalase (CAT), Glutathione Peroxidase (GPX), Reduced Glutathione (GSH), Glutathione Reductase (GR) activites in serum were estimated. The results revealed a dose dependent antidiabetic effect with 200 mg/kg body weight possessing significant activity without any toxic effect on liver and kidney. The extract treated groups were compared with that of diabetic control and standard (glibenclamide).

Key words: *Microcosmus exasperatus,* Ascidian, Antidiabetic activity and Glibenclamide.

1. INTRODUCTION

Diabetes mellitus is a major endocrine disorder affecting nearly 10% of the population all over the world [1]. This is characterized by polyuria, polyphagia, polydypsia, ketosis, retinopathy and cardiovascular disorders. The relative or absolute deficiency in the insulin level causes disturbance in the carbohydrate, fat and protein metabolism [2]. No effective chemotherapy is available to cure diabetes in modern medicine [3]. Moreover most of the drugs in use have side effects like hepatotoxicity, abdominal pain, flatulence, diarrhea and hypoglycemia [4, 5]. Hence there is an increasing demand in the use of natural products with antidiabetic activity [6, 7]. Ascidians are marine sedentary animals with pharmacological activities like antimicrobial [8, 9], antipyretic and analgesic properties [10]. They exhibit the presence of bioactive compounds with antioxidant [11] and antitumor potential [12]. A review of literature shows that studies on the nutritive value [13] of *Microcosmus exasperatus* have been reported but work on antidiabetic activity has not been attempted so far.

2. MATERIALS AND METHODS

2.1. Animal material

Microcosmus exasperatus was collected from Tuticorin harbour area and identified using key to identification of Indian ascidians [14]. A voucher specimen AS 2240 has been deposited in the museum, Department of Zoology, A.P.C. Mahalaxmi College for Women, Tuticorin - 628002. They were cleaned with sea water, shade dried and homogenized to get a coarse powder which was stored in an airtight container and used for further investigations.

2.1.1. Systematic position

Phylum: Chordata, Sub phylum: Urochordata, Class: Ascidiacea, Order: Pleurogona, Suborder: Stolidobranchia, Family: Pyuridae, Genus: *Microcosmus*: Species: *exasperatus*.

2.2. Preparation of extract

100 gm powder was extracted with ethanol using Soxhlet apparatus, cooled to room temperature and evaporated in a rotary evaporator to get a residue.

2.3. Experimental animal

Mature adult male Wistar albino rats weighing about 180 - 200 gm were selected for the study. They were maintained in a well ventilated animal house with constant 12 hours of darkness and 12 hours light schedule, room temperature (24±2 ºC) and humidity (60 - 70 %). Clean water and standard pellet diet “ad Libitum” (Hindustan Lever Ltd., India) were given to them.

The animals were kept under fasting for 16 hours before the experiment.

2.4. Induction of diabetics in experimental animal

The rats were injected with alloxan monohydrate dissolved in sterile normal saline at a dose of 150 mg/kg. 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 hypoglycemia [15]. After a fortnight rats with moderate diabetes having glycosuria (indicated with Benedict's test for urine) and hyperglycemia with blood glucose range of 200 – 260 mg/100 ml were used for the experiment.

2.5. Experimental protocol

In the present investigation non-diabetic control rats and diabetic induced rats were used. The rats were divided into five groups of six animals each. The experiment was carried out for 14 days and all the drugs were administered intraperitoneally. Group I served as normal and Group II as diabetic control. Both were given normal saline. Group III and IV diabetic rats administered with 100 and 200 mg/kg of the ethanol extract of *Microcosmus exasperatus*. Group V received the standard drug glibenclamide. At the end of experiment 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. All the experiments were conducted in accordance with the guidelines established by the animal ethics committee.

2.5.1. 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.

2.5.2. Estimation of insulin, glucose, urea, creatinine and glycosylated haemoglobin

Serum was analysed for insulin by ELISA [16], glucose [17], urea [18], creatinine [19] by colorimetric and glycosylated haemoglobin [20] was measured by using the standard methods.

2.5.3. Estimation of protein, albumin, globulin, SGPT, SGOT, ALP

Protein content was determined by Lowry method [21]. Serum albumin, globulin [22], SGPT, SGOT [23] and ALP was estimated by the procedure of King and Armstrong [24].

2.5.4. Estimation of lipids and lipoprotein

Standard instructions were followed to measure TC [25], TG [26], LDL-C, VLDL-C [27], HDL-C [28] and phospholipid [29] levels.

2.5.5. Estimation of LPO, SOD, CAT, GPx, GSH and GR

Analysis of LPO [30], SOD [31], CAT [32], GPx [33], GSH [34] and GR [35] were carried out with the serum as mentioned.

2.6. Statistical analysis

The results are expressed as means of ±SEM. The Statistical significance was determined by One Way Analysis of Variance followed by student't'-test. $P < 0.05$ was considered to be statistically significant.

3. RESULTS AND DISCUSSION

Table 1 illustrates the effect of ethanol extract of *Microcosmus exasperatus* (100, 200 mg/kg) 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 decreased after 180 min in diabetic control. In the groups treated with the extract a significant reduction in blood glucose in a dose dependent manner was observed from 60 minutes onwards. 25 and 47 percentage of reduction in blood glucose level was seen in the treated groups. According to Khan and Shechter [36], a 25 percentage reduction can be considered to have potential hypoglycemic effect. Maintenance of blood glucose level in the treated groups indicates the effectiveness of the extract.

The blood parameters such as insulin, glucose, urea, creatinine and Glycosylated haemoglobin are represented in table 2. Insulin level was significantly increased in group III and IV treated with extract (11.14±1.26, 16.56±1.87) compared to group II diabetic control (4.27±1.04). The glucose, urea and creatinine level of groups treated with 100 and 200mg/kg showed a dose related decrease in comparison with diabetic control. A significant decrease in the level of glycosylated hemoglobin in group III and IV (4.63±0.04, 3.74±0.09) compared with group II (10.89±0.01) was observed. The normalization of glucose and insulin may be due to the increase in peripheral utilization of glucose or by stimulating the secretion of insulin by the intact β cells as has been suggested with the alcoholic and aqueous extracts of the bark of *Ficus racemosa* [37]. The blood urea and creatinine level indicates a normal function and nontoxic effect on the kidney. HbAlc is used as a reliable marker to estimate the

Table -1: Effect of ethanol extract of *Microcosmus exasperatus* on oral glucose tolerance at different time points

Groups	Blood Glucose levels (mg/dl)			
	Initial	1st hour	2nd hour	3rd hour
Group I	76.57±1.78	96.26±2.11	158.37±4.73	93.49±3.16
Group II	186.35±4.64	224.79±3.86	241.66±5.81	214.69±3.52
Group III	168.68±3.59	138.22±1.81*	131.57±2.17*	126.84±1.95*
Group IV	173.56±2.67	119.58±2.48*	101.22±1.35**	91.63±1.37**
Group V	163.29±1.33	128.76±1.53*	112.37±1.55**	95.39±1.47**

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

Table -2: Blood parameters in normal and alloxan induced diabetic rats after treatment with ethanol extract of *Microcosmus exasperatus*

Parameter/ Groups	Insulin (MIu/ml)	Glucose (mg/dl)	Urea (mg/dl)	Creatinine (mg/dl)	HbA1c (%)
Group I	17.36±1.32	79.33±3.26	16.56±1.14	0.76±0.19	2.93±0.06
Group II	4.27± 1.04	214.54±11.26	29.14±1.89	1.31±0.24	10.89±0.01
Group III	11.14± 1.26*	136.34±8.43*	17.16±1.41** a	1.24±0.13*	4.63±0.04**
Group IV	16.56± 1.87** a	103.16±6.93** a	15.27±1.93** a	1.16±0.75**	3.74±0.09** a
Group V	18.35± 1.49**	71.54±5.29**	14.32±1.77**	1.03±0.67**	3.16±0.03**

Data represented as mean ±SEM, (N=6). Significance between *Diabetic control and extract treated group.
* P < 0.05, ** P < 0.01, aStandard drug and extract treated a P < 0.05

Table -3: Effect of the ethanol extract of *Microcosmus exasperatus* on protein, albumin, globulin, SGPT, SGOT and ALP

Parameter/ Groups	Protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	SGPT (u/l)	SGOT (u/l)	ALP (u/l)
Group I	7.89±0.56	4.19±0.14	3.7±0.13	18.14±0.19	20.14±0.26	174.59±6.76
Group II	5.14±0.15	3.04±0.11	2.01±0.31	29.13±0.24	31.59±0.14	209.33±9.39
Group III	5.98±0.23	3.56±0.37	2.42±0.26 a	14.16±0.26 * a	23.61±0.56 **	183.26±8.27 *
Group IV	7.43±0.13 ** a	4.39±0.46 ** aa	3.04±0.11 ** a	16.27±0.14 ** a	19.11±0.24 ** a	168.56±7.91 ** a
Group V	7.04±0.18 **	4.34±0.63 **	2.70±0.14 *	15.36±0.14 *	21.59±0.13 **	179.23±6.89 **

Data represented as mean ±SEM, (N=6). Significance between *Diabetic control and extract treated group.
* P < 0.05, ** P < 0.01, aStandard drug and extract treated a P < 0.05

Table -4: Effect of the ethanol extract of *Microcosmus exasperatus* on lipid parameters

Parameter/ Groups	TC (mg/dl)	TG (mg/dl)	LDL-C (mg/dl)	VLDL (mg/dl)	HDL-C (mg/dl)	PL (mg/dl)
Group I	86.36±2.94	72.51±1.93	35.72±1.56	14.50±0.45	36.14±1.26	144.86±2.67
Group II	189.29±3.56	214.51±4.56	90.13±1.31	42.90±1.21	56.26±8.39	236.46±2.41
Group III	123.31±2.91 *	166.34±3.93 *	33.85±1.56 **	33.26±0.93	56.20±3.84	177.74±2.64 *
Group IV	112.68±3.14 **a	126.30±2.16 **a	46.03±1.22 **a	25.26±0.28 *a	41.39±1.68 *a	168.28±2.11 **a
Group V	103.26±3.68 **	112.66±2.83 **	42.4±1.05 **	22.53±0.18 *	38.33±1.12 *	159.90±2.60 **

Data represented as mean ±SEM, (N=6). Significance between *Diabetic control and extract treated group.
* P < 0.05, ** P < 0.01, [a]Standard drug and extract treated [a] P < 0.05

Table -5: Effect of the ethanol extract of *Microcosmus exasperatus* on the level of antioxidant enzymes in plasma

Parameter / Groups	LPO (mmol/ml)	SOD (u/gm Hb)	CAT (u/gm Hb)	GPX (U/L)	GSH (mmol/ml)	GR (U/L)
Group I	1.39±0.071	453.19±32.66	71.64±3.88	719.61±34.14	36.16±1.93	23.26±1.56
Group II	4.14±0.034	287.26±26.33	43.08±2.14	302.94±36.94	17.18±1.14	13.08±0.93
Group III	2.26±0.013 *	319.18±27.69	62.14±1.93 *	583.18±29.11 *	24.93±1.39 *	16.29±0.78
Group IV	1.81±0.027 **a	411.36±39.36 **a	83.14±2.89 *a	674.28±32.33 **a	37.33±1.27 **a	26.55±0.36 **a
Group V	1.23±0.014 **	463.81±36.94 **	66.16±2.90 *	698.01±27.84 **	39.91±1.63 **	19.43±0.69 **

Data represented as mean ±SEM, (N=6). Significance between *Diabetic control and extract treated group.
* P < 0.05, ** P < 0.01, [a]Standard drug and extract treated [a] P < 0.05, [aa] P < 0.01

reaction between excess glucose in blood and free amino groups of globulin indicated by protein glycation [38]. Administration of the extract significantly reduced the HbAlc level due to normoglycemic control mechanism as suggested in some plants [39]. As ascidians are sedentary like the plants the same role can be attributed to the extract.

The total protein, albumin and globulin content showed a dose dependent increase whereas the level of SGPT, SGOT and ALP decreased in Group III and IV compared to the diabetic control as shown in table 3. Insulin deficiency leads to a decrease in protein content [3]. In the present study total protein, albumin and globulin was restored to normal in all the groups treated indicating a normal secretion of insulin and antidiabetic effect. After treatment with ethanol extract of *Microcosmus exasperatus,* the serum enzymes were brought to normal values. The restoration of SGPT, SGOT and ALP to their respective normal level as an indication of normal function of liver has been reported in plants by earlier workers [40, 41]. Change in the level of serum enzymes are also directly related to change in metabolism indicating improvement in glucose metabolism.

Table 4 indicates the serum lipid parameters like TC, TG, LDL-C, VLDL, HDL-C and PL. These parameters showed a dose dependent decrease compared to that of diabetic control. The different parameters studied were higher when compared to that of normal control whereas the values were more close to the group treated with the standard drug. An increased cholesterol level leads to serious pathological condition. It is suggested that in the present study a higher dose of 250mg/kg may decrease the lipid parameters to normal. An increase in serum concentration of TC, TG, LDL-C, VLDL, HDL-C and PL in diabetic control is linked with hyperlipidaemia[42, 43]. Insulin deficiency inactivates lipoprotein lipase which converts free fatty acids into phospholipids, cholesterol and releases into blood [44, 45]. The

treatment with extract for 14 days showed a significant (P< 0.01) fall in lipid profile. This is indicative that *Microcosmus exasperatus* may possess insulin like activity.

The level of antioxidant enzymes in plasma is shown in Table 5. Lipid peroxide level showed a decrease where as 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 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 [39]. A significant elevation in the level of all other enzymes on treatment with the extract proves that it possess antioxidant properties.

4. CONCLUSION

To conclude it is suggested that the ethanol extract of *Microcosmus exasperatus* shows antidiabetic properties in animal model. A preliminary chemical screening has revealed the presence of terpenoids, alkaloids, flavonoids, saponins, phenols and a GC-MS analysis [46] showed compounds like 2-Piperidinone, Benzeneacetamide, n-Hexadecanoic acid and 3-pentadecyl-Phenol having antioxidant activity which are known to enhance free radical scavenging leading to antidiabetic effect. Detailed studies on isolation, characterization and structure determination of the exact chemical compounds could lead to pharmacologically potent drug molecules.

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