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Antidiabetic activities of foliar application of extract of *Ulva reticulata* grown fruit of *Cyamopsis tetragonoloba* (L.) taub. in alloxan-induced diabetic rats

Abstract

Green leafy and fruit vegetables have been commonly used in the treatment of various human diseases and are considered as alternative medicines in the current scenario. In the present study, the methanolic extract of *Ulva reticulata* grown fruit of *Cyamopsis tetragonoloba* was investigated against alloxan induced diabetic rats. Wistar albino rats were divided into seven groups and allowed to acclimatize for 7 days. Diabetes mellitus was induced in rats by single intraperitoneal injection of Alloxan (150mg/kg body weight). Group I served as the normal control (positive control) which received normal saline, group II served as the diabetic induced control (reference control), group III received 600µg/kg glibenclamide which was used as reference drug, Group IV received 200mg/g extract of control fruit, group V received 400mg/g extract of control fruit, group VI received 200mg/g extract of treated fruit and group VII received 400mg/g extract of treated fruit of *Cyamopsis tetragonoloba*. Control fruit extract (200 and 400mg/kg) showed gradual blood glucose lowering effect in alloxan-induced hyperglycemic rat and the sugar level was 161.50mg/dl and 111.25mg/dl respectively lesser than the reference control (301.5mg/dl). In alloxan - induced diabetic rat administered with seaweed grown *C. tetragonoloba* (200mg/kg and 400mg/kg) exhibited 117mg/dl and 98.25mg/dl reduction of blood glucose level respectively when compared to control fruit extract. The results clearly indicated that the foliar application of extract of *U. reticulata* grown fruit of *C. tetragonoloba* obsessed antidiabetic activity against alloxan induced diabetic rats.

Keywords: diabetes mellitus, antidiabetic activity, *Cyamopsis tetragonoloba*, alloxan, glibenclamide

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Introduction

Diabetes mellitus is a clinical syndrome characterized by in appropriate hyperglycemia caused by a relative or absolute deficiency of insulin or resistance to the action of the hormone at the cellular level.[1] Currently, the world diabetes prevalence in adults above 20 years of age is over 150million and is expected to increase to 366 million by 2030.[2] India has today become the diabetic capital of the world over 20million diabetics and this number is set to increase to 57million by 2025.[3] Hyperglycemia can be handled initially with oral agents and insulin therapy, which sometimes required achieving targeted glycemic levels. However, these synthetic agents produce some serious side effects and relatively expensive for developing countries.[4] Therefore, searching for effective, low cost and less side effect hypoglycemic agents is important. Herbal remedies for diabetes are known since ancient times in different places. One such plant is *Cyamopsis tetragonoloba* is a well known traditional plant used in folklore medicine of India, expected to have hypoglycemic activity. Many researchers have shown the relationship between legume consumption and health benefits, such as protection from diabetes, cardiovascular disease and breast cancer.[5,6] The aim of the present study was to find out the antidiabetic activity of foliar application of extract of *Ulva reticulata* grown fruit of *Cyamopsis tetragonoloba* L. in alloxan induced diabetic rats.

Materials and methods

Collection of seaweed

Green alga (*U. reticulata* Forsskal) was collected during low tide, at Hare Island, Thoothukudi from November 2014 to February 2015. The sample was washed thoroughly with seawater followed by fresh water to remove sand particles and macroscopic epiphytes. After draining, the seaweed was shade-dried, powdered, sieved and used for the preparation of seaweed concentrate.

Preparation of seaweed liquid fertilizer for foliar application

Seaweed extracts (SWEs) were prepared by adopting the method of[7] with certain modifications. About 20g dried seaweed powder was mixed with 200ml distilled water and heated to 60°C. Then the mixture was maintained at the temperature for 24 hr in a hot air oven. The extract was filtered and then centrifuged at 10000 rpm to remove suspended impurities. The filtrate was stored in air tight bottles at 4°C (100% seaweed concentrate) for further use.

Experimental design

A pot culture experiment was conducted during February to April 2015 at Plant Research Centre, St. Mary's College Campus,

Thoothukudi, Tamil Nadu, India. The pots were filled with 3kg of garden soil. 20 seeds of *C. tetragonoloba* were sown in each pot. After the emergence of seedlings, they were thinned to ten plants per pot and allowed to grow up to fruiting stage. Weeding and watering were done at regular intervals. 1% SWE was applied as foliar spray (along with 100ml of distilled water in the ratio of 1:100) after expansion of first leaf and was continued till fruiting stage. Enough replicates were maintained.

Antidiabetic activity

Experimental induction of diabetes in rats: Three months old male Wistar albino rats weighing 130-150g were obtained from the animal house, Agricultural University, Trissur, Kerala. All animals were kept in an environmentally controlled room with 12 hours light/12 hours dark cycle. The animals had free access to water and standard rat diet. The rats were injected intraperitoneally with alloxan monohydrate dissolved in sterile normal saline at a dose of 200 and 400mg/kg body weight. Since alloxan is capable of producing fatal hypoglycemia as a result of massive pancreatic insulin release, rats were treated with 20% glucose solution intraperitoneally after 6 hrs. The rats were then kept for the next 24 hours on 5% glucose solution bottles in their cages to prevent hypoglycemia.[8] Then the alloxanized rats were maintained for 7 days with free access to food and water.

In the present investigation, non diabetic control rats and diabetic induced rats were used. Diabetic was induced in rats two weeks before starting the treatment. The rats were divided into the following seven groups after the induction of diabetics. Each group consists of 6 rats.

Group-I: Non-diabetic rats received normal saline daily for 7 days, orally by using an intragastric catheter tube (IGC) and served as normal control.

Group-II: Diabetic rats received normal saline daily for 7 days, orally by using an IGC, at a dose of 2.5ml/kg body weight and served as diabetic induced control.

Group-III: Diabetic rats were given glibenclamide (600µg/kg body weight) for 7 days orally by using an IGC

Group-IV: Diabetic rats were fed with fruit extract of *C. tetragonoloba* (control) at the dose of 200mg/kg body weight daily for 7 days, orally by using an IGC.

Group-V: Diabetic rats were fed with fruit extract of *C. tetragonoloba* (control) at the dose of 400mg/kg body weight daily for 7 days, orally by using an IGC.

Group-VI: Diabetic rats were administered with fruit extract of *C. tetragonoloba* (SWE treated) at the dose of 200mg/kg body weight daily for 7 days, orally by using an IGC.

Group-VII: Diabetic rats were administered with fruit extract of *C. tetragonoloba* (SWE treated) at the dose of 400mg/kg body weight daily for 7 days, orally by using an IGC.

The rats were fasted on the 8th day for 12hrs and their blood glucose levels were determined using one touch glucometer (Lifescan, Johnson and Johnson, California, USA). Rats with glucose levels above 200mg/dl were used for studying the impact of fruit extract of *C. tetragonoloba*. Blood glucose level were tested at regular interval viz., 0 day, 1 day, 3rd day, 5th day and 7th day and recorded.

Results and discussion

Diabetes is a chronic disease characterized by high blood glucose levels due to absolute or relative deficiency of circulating insulin levels. Though different types of oral hypoglycemic agents are available along with insulin for the treatment of diabetes mellitus, there is an increasing demand by patients to use the natural products with anti-diabetic activity[9] Insulin cannot be used orally and continuous use of the synthetic drugs causes side effects and toxicity. Herbal drugs are prescribed widely even when their biologically active compounds are unknown, because of their effectiveness, less side effects and relatively low cost.[10] *C. tetragonoloba* is a well known traditional plant used in folklore medicine of India, expected to have hypoglycemic activity.[11] Hence the effect of feeding (orally) methanolic pod extract of *C. tetragonoloba* was investigated on fasting blood glucose levels in glucose loaded, normal and alloxan-induced diabetic rats and compared with glibenclamide, a reference drug. Based on preliminary study, methanolic extract of *C. tetragonoloba* was found to be safe for biological studies, as no lethality was observed upto 2000 mg/kg (i.p) in rat.

Antidiabetic effect of methanolic extracts of *C. tetragonoloba* pods in alloxan induced rat is presented in Table 1. It was found that after 48 hrs of glucose administration, glucose level was rapidly increased in all the (alloxan induced) experimental groups of rat except control (Group I), thereafter group II was left as such without any treatment (reference control), III, IV, V, VI and VII groups of rat were administered with glibenclamide (600µg/kg i.p.), control plant extract (200mg/kg p.o.), control plant extract (400mg/kg p.o.), treated plant extract (200mg/kg p.o) and treated plant extract (400mg/kg p.o) respectively. Analyses revealed that blood glucose level was increased gradually in alloxan induced rat (reference control) from 254.5 mg/dl to 301.5 mg/dl (Group II). Administration of glibenclamide (600 µg/kg body weight) induced time dependent significant hypoglycemic effect. Oral administration of extract of *C. tetragonoloba* (Control 200 mg/kg body weight) showed gradual blood glucose lowering effect in alloxan-induced hyperglycemic rat and the sugar level was 46.4% lesser than the reference control. However, when the dosage was increased (400 mg/kg body weight) blood glucose level was significantly reduced (63%) in comparison with reference control. Further, it was fascinating to note that in alloxan - induced diabetic rat administered with seaweed grown *C. tetragonoloba* fruit extract, the blood sugar level was highly reduced, remarkably depending up on extract concentration (Table 1). In group VII the blood sugar level was 13% more than positive control, 67.4% less than reference control, portraying the efficacy of pods grown under seaweed extract. Reduction of blood sugar in group IV, V, VI and VII may be variously discussed (a) there are a lot of reports implicating that some phytochemical compounds in plants are being responsible for their antidiabetic activities.[12,13] Some of these phytochemical compounds revealed to be present in *C. tetragonoloba* fruit extract include phenols, flavonoids and vitamin E and minerals like magnesium may in part be responsible for the observed significant activity either singly or in synergy with one another. (b) Glibenclamide, like other sulphonylureas, is effective in mild diabetic state and ineffective in severe diabetic animals where pancreatic β-cells are completely destroyed.[14] The exogenous administration of insulin is known to produce hypoglycemia in both normal and alloxan-induced rat. It is therefore, conceivable that the hypoglycemic principle in the aqueous extract of *C. tetragonoloba*

exerted a direct effect in diabetic rat. In diabetic rat fruit extract could not act indirectly by stimulating the release of insulin since alloxan treatment causes permanent destruction of β-cells.[15]

Table 1 Ant diabetic activity of *C. tetragonoloba* fruit extract

Group	Drug and treatment	Normal blood glucose (before 48 hrs) mg/dl	Blood glucose level (after 48 hrs) mg/dl	Blood glucose level (mg/dl)				
				0 day	1st day	3rd day	5th day	7th day
I	Control (Positive)	76.00±1.82	79.25±0.95	7.75±0.95	79.00±0.81	79.00±0.81	78.00±0.81	77.5±0.57
II	Reference control (Alloxan only) (150mg/kg i.p)	77.25±1.50	243.5±5.80	254.50±5.80	264.00±5.59	274.50±5.80	286.25±6.02	301.5±4.7
III	Standard (Alloxan + Glibenclamide) (600µg/kg i.p)	72.75±0.95	243.5±8.22	248.50±6.55	206.00±2.16 (21.9)	151.25±6.80 (44.8)	106.75±4.03 (62.6)	78.00±3.36 (74)
IV	Test (Alloxan+ fruit extract of control extract- 200mg/kg p.o)	74.50±1.29	255.75±14.43	256.50±14.43	237.00±9.93 (10.2)	213.70±4.34 (22.1)	185.25±10.90 (35.2)	161.50±7.76 (46.4)
V	Test (Alloxan+ fruit extract of control extract - 400mg/kg p.o)	74.00±0.81	267.5±15.43	261.50±16.05	226.50±12.92 (14.2)	184.25±12.65 (32.8)	149.75±14.63 (47.6)	111.25±20.2 (63)
VI	Test (Alloxan+ fruit extract of treated -200mmg/kg p.o)	75.00±3.55	266.25±13.42	258.75±10.53	226.25±13.09 (14.2)*	186.50±7.22 (32)*	149.50±10.34 (47.7)*	117.00±10.67 (61.1)*
VII	Test (Alloxan+ fruit extract of treated-400mg/kg p.o)	74.50±1.29	263.00±11.91	260.75±11.98	222.50±17.67 (15.7)*	180.00±10.75 (34.4)*	141.00±15.29 (50.7)*	98.25±10.65 (67.4)*

Methanolic fruit extracts were used for analysis. Values are the mean of four replicates ±standard deviation.

Group IV and V-Control = Plants irrigated with water.

Group VI and VII-Treated = Extract of U. reticulata (1%) was applied as foliar spray until fruit setting.

Values within parentheses indicate percentage reduction in blood glucose level.

*denotes $p<0.05$ comparison made between control and treated.

Conclusion

The study revealed that the foliar application of extract of *U. recticulata* grown fruit of *C. tetragonoloba* have the hypoglycemic effect in alloxan induced diabetic rats due to presence of novel components. However, further studies need to identify the active principles responsible for the hypoglycemic effect. This will be used as an organic remedy for diabetes.

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Conflict of interest

The author declares that there is no conflict of interest.

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