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Anticholesterolemic Activity of Foliar Application of Extracts of Ulva Reticulata Grown Fruit of Cyamopsis Tetragonoloba (L) Taub in Alloxan Induced Diabetic Rats

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Abstract: - The methanolic extract of foliar application of extracts of Ulva reticulata grown fruit of Cyamopsis tetragonoloba was investigated for its anticholesterol effect in alloxan induced rats. Group I represented control, group II, III, IV, V, VI and VII represented reference control, standard Glibenclamide (600 µg/kg), control plant extract (200 mg/g), control plant extract (400 mg/g), treated plant extract (200 mg/g), treated plant extract (400 mg/g) respectively. Oral administration of control fruit extract (200 mg/kg) decreased Total Cholesterol (TC), Total Glycerides (TG), Low Density Lipoprotein (LDL), Very Low Density Lipoprotein (VLDL) and increased High Density Lipoprotein (HDL) by 19.67 %, 19.4 %, 26.42 %, 21.5 % and 23.37 % respectively. In group VI treated fruit extract (200 mg/kg) TC, TG, LDL and VLDL were reduced by 22.14%, 22.23%, 31.34% and 26.67% in comparison with reference control respectively. HDL cholesterol (32.93%) was increased in relation to reference control portraying the efficacy of seaweed extract. The result clearly indicated that extracts of Ulva reticulata grown fruit of Cyamopsis tetragonoloba acquire a considerable and dose dependent hypercholesterolemic effect in alloxan induced rats as effective as the standard drug.

Keywords: Anticholesterol effect, Glibenclamide, alloxan, seaweed extract, Ulvareticulata,Cyamopsis tetragonoloba

INTRODUCTION

Hypercholesterolemia is a major risk factor for the development and progression of atherosclerosis and related cardiovascular disease. This phenomenon is due to several factors such as unhealthy food consumption and sedentary lifestyle (Cruz 2000; Wu et al. 2012). Several modern drugs in use such as statins, fibrates, nicotinic acid and resins (Satoskar et al., 2003), lower blood cholesterol level, either by inhibiting endogenous synthesis and by lowering cholesterol absorption from the intestine (Sedaghat et al., 1975). Though many efficacious lipid-lowering synthetic drugs exist, none is effective for all lipoprotein disorders, and all such agents are associated with some adverse effects (Desu and Saileela, 2013). As a result of their side effects, people are looking for safer alternatives and the search for new drugs capable of reducing and regulating serum cholesterol level. Moreover composition of the diet also plays an important role in the management of lipid and lipoprotein concentrations in blood (Lamarche et al., 2004). Pulses form an important component of the Indian diet and have been reported to lower the cholesterol levels because of their proteins, carbohydrates and fibre content (Singh et al., 1983). Dietary fibres appear to interfere with increased excretion of cholesterol and faecal bile acids resulting in depletion of hepatic cholesterol pools and alteration in lipoprotein metabolism (Venkatesan et al., 2003). Although many research work has been done on clusterbean, this may be a novel endeavour to investigate anticholesterolemic activity of extract of Ulva reticulata grown fruit of Cyamopsis tetragonoloba 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 (Rao, 1990) with certain modifications. About 20g dried seaweed powder was mixed with 200ml distilled water and heated to 60oC. Then the mixture was maintained at the temperature for 24 hr in a hot air oven. The extract was filtered and centrifuged at 10000 rpm to remove suspended impurities. The filtrate was stored in air tight bottles at 4oC (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 Cyamopsis 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.

Preparation of fruit extracts for anticholesterol study

The extracts of U. reticulata grown fruit of C. tetragonoloba were shade dried at room temperature and the dried fruit were powdered in a Wiley mill. Hundred grams of powdered U. reticulata grown fruit of C. tetragonoloba was packed in a Soxhlet apparatus and extracted with ethanol. The methanol extracts were concentrated in a rotary evaporator. The concentrated methanol extracts were used for anticholesterol studies.

Experimental induction of diabetes in rats

Three months old male Wistar albino rats weighing 130 - 150 g 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 400 mg/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 (Dhandapani et al., 2002). 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. All animal procedures were followed in accordance with the approved protocol for use of experimental animals set by the Institutional Animal Ethical Committee. 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 2.5 ml/kg body weight and served as diabetic induced control.

Group - III : Diabetic rats were given glibenclamide (600 µg/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 200 mg/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 400 mg/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 200 mg/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 400 mg/kg body weight daily for 7 days, orally by using an IGC.

On the 8th day, blood was collected by retero orbital sinus puncture, under mild ether anaesthesia. The collected samples were centrifuged for 15 minutes at 2500rpm. Then serum samples were collected and analyzed for serum Total Cholesterol (Parekh and Jung, 1970), Triglycerides (Rice, 1970), High Density Lipoprotein Cholesterol (Burstein et al. (1970), Low Density Lipoprotein Cholesterol and Very Density Lipoprotein Cholesterol (Friedwald et al. (1972).

RESULTS AND DISCUSSION

Hyperlipidemia is a complication associated with diabetes mellitus (Miller et al., 2002) due to qualitative and quantitative abnormalities in lipoproteins. A number of epidemiological investigations have shown a clear association between dietary saturated fat and atherosclerosis (Posner et al., 1991). Moreover, many studies have shown that elevated total or low density lipoprotein (LDL) cholesterol in the blood are powerful risk factors for coronary heart disease (Law, 1999), whereas high HDL-cholesterol: LDL-cholesterol ratio may protect against coronary heart disease (Castelli et al., 1992). Several studies showed that plant extracts lowered LDL oxidation (Doi et al. 2000; Naidu and Thippeswamy, 2002). Pod of guar reduced total cholesterol, LDL and increased HDL cholesterol (Frias and Sgarbieri, 1998). The present study was designed to evaluate the effect feeding of SWE grown C. tetragonoloba fruit extract on blood lipid profile of diabetic rats. Group I represented control, group II, III, IV, V, VI and VII represented reference control, standard Glibenclamide (600 µg/kg), control plant extract (200 mg/g), control plant extract (400 mg/g), treated plant extract

(200 mg/g), treated plant extract (400 mg/g) respectively (Table 1). Analyses revealed that total cholesterol, triglycerides, LDL, VLDL were increased rapidly in alloxan induced rat (reference control) and HDL was decreased. Administration of Glibenclamide (600 µg/kg) induced time dependent anticholesterolaemic effect. Oral administration of control fruit extract (200 mg/kg) decreased TC, TG, LDL, VLDL and increased HDL by 19.67 %, 19.4 %, 26.42 %, 21.5 % and 23.37 % respectively. However, when the dosage was increased (400 mg/kg) TC (23.66%), TG (21.16%), LDL (36.35%), VLDL (23.77%) were decreased and HDL (28.27%) was increased. Further, it was noticed that in alloxan induced rat administered with seaweed grown C. tetragonoloba extract serum lipid profile was decreased remarkably depending on extract concentration except HDL cholesterol (Table 1). In group VI treated fruit extract (200 mg/kg) TC, TG, LDL and VLDL were reduced by 22.14%, 22.23%, 31.34% and 26.67% in comparison with reference control respectively. HDL cholesterol (32.93%) was increased in relation to reference control portraying the efficacy of seaweed extract. However, when the dosage was increased (400 mg/kg body weight) TC, TG, LDL, VLDL was decreased considerably. This may be due to rich source of minerals, vitamins present in the SWE grown plants than control plant. Suzuki et al. (2002) suggested that intake of vegetables and fruits rich in carotenoids might be protective against hyperlipidemic and hyperglycemia. Sarathy and Saraswathi (1983) reported that Cluster bean powder (0.5% and 2.5%) showed reduction in serum cholesterol level in 7 weeks when administered to albino rats. Pande et al. (2012) reported that cluster beans significantly decreased LDL and triglycerides but increased HDL in experimental rats. Saeed et al (2012) reported that guar gum diet significantly decreased the serum concentration of cholesterol, triglycerides and LDL-C. Several clinical researches have shown that guar gum absorption reduces the plasma cholesterol concentrations mainly due to a reduction of plasma LDL-C concentration, without affecting the HDL-C levels in normal, diabetic rats and in patients with hyperlipidemia (Uberoi et al., 1992). This result clearly indicated that SWE treated fruit exerted anticholesterol effect. The research finding enables consumers to capture the full benefits of "organic" vegetables.

Table 1. Effect of C. tetragonoloba fruit extract on serum lipid profile of alloxan induced rat

Group	Drug and treatment	TC (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
I	Control	85.12±1.25	71.68±1.80	48.10±2.09	24.4±1.92	16.03±0.98
II	Reference control (Alloxan only) (150 mg/g)	160.80±3.21	132.60±3.35	29.00±0.75	51.27±2.49	38.24±1.17
III	Standard (Alloxan + Glibenclamide) (600 µg/g)	112.80±2.95 (29.85)	94.65±1.98 (28.61)	42.12±1.63 (45.24)	28.02±3.12 (45.34)	21.81±0.78 (42.96)
IV	Test (Alloxan + control fruit extract) (200 mg/kg)	129.16±1.62 (19.67)	106.84±3.47 (19.4)	35.78±0.98 (23.37)	37.72±1.65 (26.42)	30.01±1.05 (21.5)
V	Test (Alloxan + control fruit extract) (400 mg / kg)	122.75±4.02 (23.66)	104.53±2.51 (21.16)	37.20±1.27 (28.27)	32.63±3.60 (36.35)	29.15±1.12 (23.77)
VI	Test (Alloxan + treated fruit extract) (200 mg/kg)	125.19±3.88 (22.14)*	103.42±1.83 (22.23)*	38.55±1.76 (32.93)*	35.20±2.52 (31.34)*	28.04±0.79 (26.67)*
VII	Test (Alloxan + treated fruit extract) (400 mg/kg)	119.27±2.56 (25.82)*	102.79±4.02 (22.48)*	40.45±2.14 (39.48)*	30.63±1.74 (40.25)*	26.31±1.20 (31.19)*

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 Ulva reticulata (1%) was applied as foliar spray. TC = Total cholesterol, TG = Triglycerides; HDL = High density lipoprotein, LDL = Low density lipoprotein, VLDL – Very low density lipoprotein. *p<0.05

Comparison made between control and treated. Values within parentheses indicate percentage reduction of TC, TG, LDL, VLDL and percentage increase in HDL over reference control.

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