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Original Research Article

# IMPACT OF AQUEOUS EXTRACT OF *GRACILARIA DURA* IN ALLOXAN INDUCED DIABETIC RATS.

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**Abstract:** The intake of food and control of blood glucose levels are very important in diabetic patients. To find out a potential component from seaweed, with anti-hyperglycemic effects, methanolic extract of a red seaweed *Gracilaria dura* , which contains complex mixtures of various types of bioactive compounds, which are having the hypoglycaemic effect was evaluated in alloxan-induced diabetic rats by using two different doses (200 and 400 mg $kg^{-1}$ body weight). Blood glucose, glycosylated haemoglobin, haemoglobin, serum lipids of total cholesterol, triglyceride, high-density lipoprotein cholesterol (HDL-C), phospholipids, low density lipoprotein (LDL) and plasma insulin were examined in control and seaweed extract treated experimental rats. The aqueous extract was orally administered for 28 days. The results showed that the aqueous extract of *Gracilaria dura* significantly ($p<0.05$) reduced blood glucose level and restored the glycosylated haemoglobin and plasma insulin. These results demonstrate that *Gracilaria dura* could be useful in the development of anti-diabetic drugs.

**Key words:** *Gracilaria dura*, Seaweed, alloxan, glycosylated haemoglobin, plasma Insulin, Glipizide

**Introduction**: Diabetes mellitus is a metabolic disorder in which the body does not produce or properly utilize insulin. It causes disturbance in carbohydrate, protein and lipid metabolism and complications such as retinopathy, microangiopathy and nephropathy (Zimmet *et al.* 1997). In practical terms, diabetes mellitus is a condition in diabetes, a profound alteration in the concentration and composition of lipid occurs. The global figure of people with diabetes set rise from the current estimate of 150-220 million in 2010 to 300 million in 2025(Whiting *et al.* 2011).

Despite the immense strides that have been made in the understanding and management of diabetes the disease and disease related complications are increasing unabated. In spite of the presence of known anti-diabetic medicine in the pharmaceutical market, remedies from medicinal plants are used with success to treat

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this disease (Dembinska-Kiec *et al.* 2008). Many traditional plants are used to treat diabetics throughout the world and there is an increasing demand by patients to use the natural products with anti-diabetic activity. Elavarasi *et al.* (2013) have reported that the traditional medicinal plants have hypoglycemic effects and potential effectiveness against diabetes; it is assumed that these plants have a major role to play in the management of diabetes. Many new bioactive drugs isolated from the traditional medicinal plants having hypoglycemic effects showed anti-diabetic activity equal and sometimes even more potent than known oral hypoglycemic agents.

The present investigation is undertaken to the study the effect of aqueous extract of *Gracilaria dura* on changes in body weight, plasma glucose, and hemoglobin and glycosylated hemoglobin and lipid profile.

**Experimental Models:** For the study of anti-diabetic an experimental model is selected in such a way that it would satisfy the following:

a. The animal should develop hyperglycemia rapidly.
b. Pathological changes in the site of induction should result from pancreatitis or damage of β-cells.
c. The symptoms should be ameliorated or prevented by a drug treatment effective in human beings.

## Materials and Methods

**Selection & acclimatization of animals:** Wistar strains of male albino rats weighing between 180-220gm are used for this study. The rats were housed in large spacious cages and they were fed with commercial pellets and access to water *ad libitum*. The rats were well acclimatized to the standard environmental condition of temperature ($22^0$c ± $5^0$c) and humidity (55 ±5%) and 12 hr light dark cycles throughout the experimental period.

**Induction of Diabetes Mellitus:** Diabetes mellitus is induced in wistar rats by single intraperitoneal injection of freshly prepared solution of Alloxan monohydrate (S. D Fine. Chem. Ltd, Mumbai, India) (150mg/kg BW) in physiological saline after overnight fasting for 12hrs (Al-Shamaony *et al.*1994**).**

Alloxan is commonly used to produce diabetes mellitus in experimental rats due to its ability to destroy the β-cells of pancreas possibly by generating the excess reactive oxygen species such as $H_20_2$, $0_2$ and $H0^-$. The development of hyperglycemias in rats is confirmed by plasma glucose estimation 72 hrs post alloxan injection. The rats with fasting plasma glucose level of 160-220mg/dl were used for this experiment.

**Experimental procedure:** In the experiment a total of 30 rats (24 diabetic surviving rats & 6 normal rats) were used. Diabetes was induced in rats 3 days before starting the experiment. The rats were divided into 5 groups after the induction of alloxan diabetes. In the experiment 6 rats were used in each group.

**Treatment Protocol**

| | |
|---|---|
| Group-I | Normal control: Rats given with 10ml/Kg of normal saline, orally |
| Group-II | Diabetic rats : Rats received 150mg/Kg of alloxan monohydrate |
| Group-III | Diabetic control rats received glipizide at a dose of (10mg/Kg orally) for 28 days. |
| Group-IV | Diabetic control rats received aqueous extract of *Gracilaria dura* at a dose of (200mg/Kg orally) for 28 days. |
| Group-V | Diabetic control rats received aqueous extract of *Gracilaria dura* at a dose of (400mg/Kg orally) for 28 days. |

**Methodology**

**Sample collection:** After 28 days of treatment, body weight, blood glucose, haemoglobin, glycosylated haemoglobin, plasma insulin, total cholesterol, triglycerides, HDL-cholesterol and phospholipids were determined. Blood was collected from the eyes (venous pool) by sino-ocularpuncture (Waynforth, 1980) in EDTA coating plasma tubes for the estimation of blood parameters.

**Biochemical Analysis**

**Estimation of blood glucose:** Blood glucose was estimated by commercially available glucose kit (One Touch Ultra) Johnson and Johnson based on glucose oxidase method of Trinder (1969).

**Plasma insulin:** Plasma insulin was determined by ELISA method using a Boehringer–Mannheim kit Anderson *et al.* (1993). with an ES300 Boehringer analyzer (Mannheim, Germany).

**Estimation of total haemoglobin and glycosylated haemoglobin:** Total haemoglobin was determined by the method of Drabkin and Austin (1932) and glycosylated haemoglobin was determined by the method of Sudhakar Nayak and Pattabiraman (1981).

**Estimation of lipid & lipoprotein:** Plasma lipids were determined by auto analyzer according to the method of Parkeh and Jung (1970) (total cholesterol), Gidez and Webb (1950) (HDL-cholesterol), Zilversmith and Davis (1950) (phospholipids) and Rice (1970) (triglycerides).

**Statistical analysis:** The data for various biochemical parameters were analyzed using analysis of variance (ANOVA), and the group means were compared by Newman-Keuls multiple range test (NKMRT). Values were considered statistically significant at $p<0.01$.

**Results:** Table 1 illustrates the levels of initial and final blood glucose, and change in body weight in normal and treatment control rat in each group. The mean body weight of diabetic rats (G2) was significantly decreased as compared to normal control rats. The body weight of diabetic control rats treated with *Gracilaria dura* extract at a dose of 200mg/kg and 400mg/kg was increased the body weight non-significantly as compared to normal control rats.

**Table 1: Effect of *Gracilaria dura* on initial and final body weight and blood glucose in normal and treated rats.**

| GROUP | Body weight (g) | | Blood glucose (mg / 100ml) | |
|---|---|---|---|---|
| | Initial | Final | Initial | Final |
| G1 | 225 ± 7.15 | 230 ± 7.22 a** | 85.75 ± 3.25 | 88.70 ± 3.80 a** |
| G2 | 230 ± 6.50 | 170 ± 4.45 b** | 84.60 ± 3.08 | 215.50 ± 6.54 b** |
| G3 | 225 ± 6.30 | 232 ± 6.30 c** | 88.40 ± 3.65 | 120.30 ± 4.08 c** |
| G4 | 220 ± 6.10 | 230 ± 6.40 c** | 86.80 ± 3.75 | 130.50 ± 4.25c** |
| G5 | 218 ± 6.05 | 235 ± 6.45 c** | 86.45 ± 3.60 | 126.60 ± 4.20c** |

Mean±SEM (n=6). Different letters indicate significant differences between control (a) vs. diabetic (b) and treated groups (c). **P<0.001, significant probability value

Fasting blood glucose level was significantly increased 215.50 ± 6.54 in diabetic rats as compared to normal rats. However the level of fasting blood glucose returned to near normal range in diabetic rats treated with *Gracilaria dura* extract at a dose of 200mg/kg and 400mg/kg. Table 2 illustrates the levels of total hemoglobin, glycosylated hemoglobin and plasma insulin in normal rat and treatment control rats in each group.

**Table:2-Effect of *Gracilaria dura* on plasma insulin, Hemoglobin & Glycosylated hemoglobin in normal and treated rats.**

| GROUPS | Haemoglobin (gm/100ml) | Glycosylated haemoglobin $HbA_1$ (%) | Plasma Insulin (µU/ml) |
|---|---|---|---|
| **G1** | 12.10 ± 1.65a** | 0.35 ± 0.06a** | 33.20 ± 2.70a** |
| **G2** | 6.55 ± 0.55b** | 0.95 ± 0.12b** | 12.10 ± 1.55b** |
| **G3** | 11.25 ± 1.30c** | 0.40 ±0.06c** | 27.50 ± 2.35c** |
| **G4** | 10.85 ± 0.90c** | 0.44±0.09c** | 24.40 ±2.05c** |
| **G5** | 11.05 ± 1.20c** | 0.48 ±0.05c** | 26.30 ± 2.10c** |

Mean±SEM (n=6). Different letters indicate significant differences control (a) vs. diabetic (b) and treated groups (c). **P<0.001, significant probability value.

The levels of total hemoglobin and plasma insulin levels were decreased significantly where as glycosylated heamoglobin levels were increased significantly as compared to normal control rats. However the level of total hemoglobin, glycosylated hemoglobin and plasma insulin, returned to near normal range in diabetic rats treated with *Gracilaria dura* extract at a dose of 200mg/kg and 400mg/kg

Table 3 shows the level of serum total cholesterol (TC), triglycerides (TG), high density lipoprotein (HDL), Low density lipoprotein (LDL) and phospholipids of normal and experimental rats in each group.

**Table 3. Serum lipids of Normal and experimental groups.**

| GROUPS | Total Cholesterol (mg/dl) | Triglyceride (mg/dl) | HDL-C (mg/dl) | Phospholipids (mg/dl) | LDL (mg/dl) |
|---|---|---|---|---|---|
| G1 | 83.45 ± 2.40 a** | 86.60 ± 2.40 a** | 52.35 ± 1.75 a** | 125.50 ± 2.50 a** | 15.30 ± 1.30 a** |
| G2 | 215.30± 6.75 b ** | 156.70 ± 4.25 b ** | 30.70 ± 1.35 b ** | 210.40 ± 6.30 b ** | 38.70 ± 2.40 b ** |
| G3 | 115.80 ± 3.15 c ** | 94.90 ± 2.65 c ** | 44.50 ± 1.50 c ** | 150.55 ± 3.90 c ** | 24.35 ± 1.95 c ** |
| G4 | 126.50 ± 3.60 c ** | 108.30 ± 2.90 c ** | 39.50 ± 1.40 c ** | 158.70 ± 4.15 c ** | 30.30 ± 1.92 c ** |
| G5 | 120.40 ± 3.25 c ** | 98.35 ± 2.25 c ** | 40.50 ± 1.58 c ** | 146.30 ± 3.70 c ** | 26.40 ± 1.65 c ** |

Mean±SEM (n=6). Different letters indicate significant differences control (a) vs. diabetic (b) and treated groups (c). **P<0.001, significant probability value.

Total cholesterol, triglycerides, high density lipoprotein, Low density lipoprotein (LDL) and phospholipids levels were significantly increased, where as HDL-C level was decreased in alloxan induced diabetic rats as compared to normal rats. Treatment of normal and alloxan induced diabetic rats with *Gracilaria dura* extract at a dose of 200mg/kg and 400mg/kg for 28 days resulted in marked decrease in total cholesterol, triglycerides, Low density lipoprotein(LDL) and phospholipids levels and increase in HDL-C levels as compared to alloxan induced diabetic rats.

**Discussion:** Alloxan causes massive reduction in insulin release, through the destruction of β-cells of the islets of Langerhans. The mechanism of alloxan action was fully described elsewhere (Lazarow, 1964; Colca *et al.*, 1983). In our study, we have observed a significant increase in the plasma insulin level when alloxan induced diabetic rats were treated with *Gracilaria dura* powder at a dose of 200mg/kg and 400mg/kg

this could be due to potentiation of the insulin effect of plasma by increasing the pancreatic secretion of insulin from existing β- cells of islets of Langerhans or its release from bound insulin.

In uncontrolled or poorly controlled diabetes there is an increased glycosylation of a number of proteins including haemoglobin and α-crystalline of lens (Alberti and Press, 1982). Glycosylated haemoglobin ($HbA_1C$) was found to increase in patients with diabetes mellitus to approximately 16% (Koenig *et al.*, 1976) and the amount of increase is directly proportional to the fasting blood glucose level (Jackson *et al.*, 1979). During diabetes the excess glucose present in blood reacts with haemoglobin. Therefore, the total haemoglobin level is decreased in alloxan induced diabetic rats (Sheela and Augusti, 1992). Administration of *Gracilaria dura* extract at a dose of 200mg/kg and 400mg/kg for 28 days prevents a significant elevation in glycosylated haemoglobin thereby increasing the level of total haemoglobin in diabetic rats. This could be due to the result of improved glycaemic control produced by *Gracilaria dura* extract at a dose of 200mg/kg and 400mg/kg.

The body weight was decreased in alloxan diabetic rats. Administration of *Gracilaria dura* extract at a dose of 200mg/kg and 400mg/kg increases the body weight in alloxan induced diabetic rats. The ability of *Gracilaria dura* extract at a dose of 200mg/kg and 400mg/kg to protect massive body weight loss seems to be due to its ability to reduce hyperglycemia.

The level of serum lipids are usually elevated in diabetes mellitus, and such an elevation represents the risk of coronary heart disease (CHD). Lowering of serum lipids concentration through diet or drug therapy seems to be associated with a decrease in the risk of vascular disease. The abnormal high concentration of serum lipids in diabetic subject is mainly due to increased mobilization of free fatty acids from the peripheral fat depots, since insulin inhibits the hormone sensitive lipase. However, glucagon, catecholamines and other hormones enhance lipolysis. The marked hyperlipidaemia that characterized the diabetic state may therefore be regarded as a consequence of the uninhibited actions of lipolytic hormones on the fat depots.

In the alloxan-induced diabetes mellitus, the rise in blood glucose is accompanied by an increase in serum cholesterol and triglycerides. The levels of cholesterol and triglycerides and Low density lipoprotein (LDL) levels were brought to near normal by the treatment with Gracilaria *dura* extract at a dose of 200mg/kg and 400mg/kg in alloxan induced diabetic rats.

The effect of *Gracilaria dura* extract at a dose of 200mg/kg and 400mg/kg on diabetic hypertriglyceridemia could be through its control of hyperglycaemia. This is in agreement with the facts that:

1. The level of glycaemic control is the major determinant of total and very low density lipoprotein (VLDL), triglyceride, concentrations ( Laakso , 1995).
2. Improved glycemic control following sulfonylurea therapy decreases the levels of serum VLDL and total triglycerides (Taskinen *et al.*, 1986)

The main ‘anti-atherogenic’ lipoprotein (HDL) is involved in the transport of cholesterol from peripheral tissues into liver (Segal *et al.* 1984) and thereby it acts as a protective factor against coronary heart disease (CHD) Gordon *et al.*, 1977).

The level of HDL-cholesterol was decreased in diabetic rats when compared with normal rats (Pushparaj *et al.*, 2000). Our results clearly show that the level of HDL-cholesterol was increased in alloxan induced diabetic rats when treated with *Gracilaria dura* extract at a dose of 200mg/kg and 400mg/kg. These results suggest that *Gracilaria dura* extract at a dose of 200mg/kg and 400mg/kg has protective effect against alloxan-induced diabetes and its complications.

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