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ScienceDirect

Food Science and Human Wellness 5 (2016) 57–64

Food Science and Human Wellness

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# The protective effect of dietary flavonoid fraction from *Acanthophora spicifera* on streptozotocin induced oxidative stress in diabetic rats

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Received 10 December 2015; received in revised form 31 January 2016; accepted 13 February 2016
Available online 21 February 2016

**Abstract**

The present investigation was considered in arraying of antidiabetic and antioxidant activity from dietary flavonoid loaded fraction of *Acanthophora spicifera* (*A. spicifera*, Family: Rhodomelaceae) on streptozotocin (STZ) induced oxidative stress rats. The testings were acted upon male rats, which were alienated into five groups: control group, diabetic group (single dose of 65 mg/kg, streptozotocin (STZ) i.p.), diabetic with insulin (6 IU), and diabetic with flavonoid rich fraction groups (FRF) at 50 and 100 mg/kg body weight, given orally for 21 days. The blood glucose level was determined at different week intermissions. The antioxidant consequences of FRF on STZ-induced diabetic rats were determined by the estimations of the oxidative stress marker like malonyldialdehyde and antioxidant enzymes such as superoxide dismutase, catalase and glutathione in tissue homogenates of heart, liver and kidney. FRF treatment of diabetic rats significantly ($P < 0.05$) diminishes the blood glucose altitudes to normal in contrast with diabetic rats. However, FRF administration, significantly decreased the malonyldialdehyde (MDA) and increased the activities of superoxide dismutase (SOD), catalase (CAT) and glutathione levels (GSH) in diabetic rats. The outcome designates that FRF fraction from red algae *A. spicifera* was potent anti diabetic and antioxidant asset against STZ induced diabetes and oxidative tissue breakups.


*Keywords:* *Acanthophora spicifera*; Red algae; Streptozotocin; Diabetes; Antioxidant

## 1. Introduction

Diabetes mellitus is a metabolic ailment, as old as mankind and its occurrence is considered to be far above the ground at all over the world [1]. It is also a major root of disability and hospitalization, results in a significant pecuniary burden [2]. The management of diabetes mellitus is painstaking and thriving cure is yet to be discovered. The circumstances were primarily grim in budding countries like India, where unprecedented economic growth has been accompanied with an unfortunate byproduct of that prosperity in the form of diabetes [3,4]. It was sturdily supported by greater than ever incidences in both experimental and clinical swot. It puts forward that oxidative stress caused by hyperglycemia, plays a foremost role in pathogenesis of diabetes mellitus (DM). Diabetes is habitually accompanied by hyperglycemia with large amplification of reactive oxygen species (ROS) and impaired antioxidant defense co-ordination [5,6]. Oxidative stress, as an episode of oxidant factors over antioxidant mechanisms, plays an innermost role in the pathogenesis and progression of diabetes and its complications. Hence, it is likely that a stuff known to reduce oxidative stress *in vivo* would trim down the progression of cell damage in clinical diabetes. A dietery flavonoids (abundant in plants, vegetables and Fruits) has

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Peer review under responsibility of Beijing Academy of Food Sciences.

http://dx.doi.org/10.1016/j.fshw.2016.02.002

been accounted to have a number of potentially beneficial effects in both hindrance and management of oxygen-related diseases: for example, the enhancement of glucose utilization in type II diabetes [7] and reduction of the development of diabetic hitches [8]. Investigations in this particular region are now sparkling insights of potential benefits in diabetes. Seaweeds have traditionally been used as foodstuff and folk remedy for helminthes infections, gout and eczema, predominantly by coastal working class in several countries [9]. Recently, much attention has been paid to the anticancer tumult of seaweeds due to their rich phyto-constituents and has reported that crude Swedes or their organic extracts have an influence on pancreatic β-cells [10].

The seaweed *Acanthophora spicifera* (Family: Rhodomelaceae, Ceramiales) is notorious red algae and widely distributed in the Gulf of Mannar, Rameshwaram coast, Tamilnadu, South India which is used as a food material, cosmetics, and fuel [11]. Besides, methanol extract of *A. spicifera* has been an evidence for anti-bacterial activity against *Staphylococcus aureus* and *Bacillus subtilis* [12]. The sulphated agarans are isolated from the aqueous extract of *A. spicifera* and their anti-viral chattels with their structure activity relationship were recorded [13]. Antioxidant properties of *A. spicifera* were deliberate in different types of *in vitro* free radical scavenging assays [14]. Hence, present study was designed to extend the current information on anti-diabetic folklore claim and antioxidant effect of *A. spicifera* and to determine its protective effects on rats having uncontrolled type I diabetes.

## 2. Materials and methods

### 2.1. Marine algae collection

The red algae, *A. spicifera* (Family: Rhodomelaceae, Ceramiales) was collected from Mandapam, during the month of March, 2014 from Rameswaram coast, Tamil Nadu, India [15]. It was identified and authenticated by Dr. Krishnamurthy, Institute of algology, Annanagar, Chennai. The voucher specimen (SVCOP/14-125) was deposited in the Department Museum.

### 2.2. Preparation of the ethanol extract and separation flavonoids rich fraction from Acanthophora spicifera

Dried, milled *A. spicifera* (1 kg) was extracted through 5 l of ethanol by means of soxhlet apparatus for 24 h. The extract was filtered, and the filtrate was evaporated by a rotary vacuum evaporator. The gain in yield of the menthol extract was found to be 20.22% (w/w). The dried ethanol extract was suspended in water and assorted with n-hexane in a separating funnel and n-hexane portion was discarded after separation. To the aqueous portion, dichloromethane was added and the dichloromethane portion was discarded after separation and the aqueous portion was collected and further extracted with ethyl acetate. The ethyl acetate portion was collected and it was allowed to dry for complete removal of solvent by a rotary vacuum evaporator. The yield of the ethyl acetate fraction was 2.45% (w/w). The ethyl acetate fraction was subjected to qualitative chemical test and thin layer chromatography (TLC) studies and showed positive test for flavonoids.

### 2.3. Estimation of total phenol content in FRF

Total phenolic content was estimated with Folin–Ciocalteu colorimetric method described previously [16,17] with a little modification. Briefly, the appropriate dilutions of the FRF were oxidized with 0.2 N Folin–Ciocalteu reagents and then the reaction was neutralized with saturated sodium carbonate (75 g/l). The absorbance of the resulting blue color was measured at 760 nm after incubation for 2 h at 23 °C. Quantification was done on the basis of the standard curve of Gallic acid. Results were expressed as grams of gallic acid equivalent (GAE) per 100 g of dry weight (DW).

### 2.4. Animals

Healthy adult Wistar rats of either sex, weighing 200–250 g, were used. The animal room was maintained at 22 ± 5 °C with a daily light-dark cycle (06:00–18:00 light) and humidity about 50%–60%. Animals were given food and water *ad libitum*. All the studies were conducted in accordance with the Animal Ethical Committee (SVCOP/02/2015/SV0026).

### 2.5. Acute oral toxicity study

Acute toxicity was carried out according to the Organization for Economic Co-operation and Development guidelines (OECD 423). Two groups of control rats ($n = 3$ in each group) were given FRF separately 2000 mg/kg p.o. as single dose. After oral administration animals were observed continuously for 2 h for beneath profiles like alertness, restlessness, irritability, fearfulness spontaneous activity, reactivity, touch response, pain response, defecation and urination. After the stage of 24 and 72 h, animals were observed for signs of lethality or for death.

### 2.6. Evaluation of oral glucose tolerance test (OGTT)

Initial screening of fractions for hypoglycemic activity was carried out in normal healthy rats by conducting OGTT. The OGTT was performed for two different doses of FRF (50 and 100 mg/kg bodyweight per orally) and blood glucose level was measured by one touch glucometer (Accu-check, India). The glucose level was measured at the intervals of 0, 30, 60, 90 and 120 min after the administration of test samples [18].

### 2.7. Induction of diabetes in rats

Diabetes was induced by a single intra-peritoneal injection of newly prepared streptozotocin (65 mg/kg) in 0.1 mol/L citrate buffer (pH 4.5) to overnight fasted rats. In order to prevent initial drug induced hypoglycemia, STZ injected animals were given with 5% glucose water for 24 h. After three days of STZ administration, rats were divided according to their fasting blood glucose levels which showed >300 mg/dl. The animals did not show the above blood glucose range is excluded from the study [19].

### 2.8. Experimental design and drug administration

Rats were divided in to four groups of six rats in each group. Group I (normal animals) treated with only vehicle, 0.3% CMC