



Effect of *Syzygium Cumini* (l) Seed Extract on Carbohydrate, Non Enzymatic Antioxidants and Anti Oxidative Enzymes in Alloxan Induced Diabetic Rats

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ARTICLE INFO

Article history:

Received: 13 February 2015;

Received in revised form:

15 March 2015;

Accepted: 7 April 2015;

Keywords

Anti-oxidative enzymes,
Antioxidant enzymes,
Diabetes mellitus,
Syzygium cumini.

ABSTRACT

In the present study oral administration of ethanol extract of *Syzygium cumini* seed from fruit to Alloxan monohydroxide induced diabetic rats secluded the rats from the changes induced in carbohydrate and enzymatic studies were seen. The selected *Syzygium cumini* (L) plant seed extract (100 and 200 mg/kg bw) seems to be more efficient in the control of type II diabetes. Oral administration of ethanol *S.cumini* extract able to control the diabetes induced alterations in enzymes related to carbohydrate metabolism. Non enzymatic antioxidant enzymes like ascorbate, Vitamin C, Vitamin E and TTH and antioxidant defense enzymes like SOD, CAT and GPx levels also regulated by the supplementation of *S. cumini* seed extract to the alloxan induced rats. The reduction in carbohydrate and enzymatic levels in diabetic rats can be used as a marker in the evaluating the severity of diabetes.

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Introduction

Globally we are facing an epidemic of non-communicable diseases, which will soon surpass communicable diseases both in the developing and the developed world. [1] Diabetes mellitus, once considered a disease of minor significance to world health, is now a major threat to human health in the 21st century. [2] A recent study by the World Health Organization (WHO) estimated that the worldwide prevalence of diabetes in 2002 was 170 million, with the number predicted to grow to 366 million or more by 2030. The majority of this diabetic population will emerge from developing countries. [3] Many synthetic oral hypoglycemic agents like Sulphonylureas, biguanides, thiazolidinediones, meglitinide derivatives and α -glucosidase inhibitors are presently in use but they all have several side effects [4]. Most of the plants contain glycosides, alkaloids, terpenoids, flavonoids, carotenoids, etc., that are frequently implicated as having antidiabetic effect [5]. This necessitates the use of herbal preparations, plant decoctions or infusions, for their little side effects, easy availability and cost effectiveness. Hypoglycemic activity of the plants is mainly due to their ability to restore the function of pancreatic tissues by causing an increase in insulin output or inhibit the intestinal absorption of glucose or to the facilitation of metabolites in insulin dependent processes. Despite the availability of various classes of antidiabetic agents, diabetes mellitus remains a major cause of mortality and morbidity globally [6]. As a result, there has been a considerable effort to search for more effective drugs. This has resulted in a renewed interest in research that investigates the health benefits of herbs and natural products including *Syzygium cumini* in the management of diabetes mellitus. *Syzygium cumini* (L) (Myrtaceae) is a medicinal plant locally Telugu name as "neredu" and it is also called as *Eugenia jambolona*, Jamun, Black plum and Indian black berry. It is a large ever green tree up to 30 m high, the leaves measuring about 10 to 15 cm long and 4 to 6 cm wide. These are entire, ovate-oblong, sometimes lanceolate and also acuminate, coraceous, tough and smooth with shine above. It is widely distributed throughout India. It has been valued in Ayurveda and Unani system of medication for possessing Variety of therapeutic[7]. In present study, we

evaluate the hypoglycemic activity of *Syzygium cumini* seeds. The present work was premeditated with seeds as the test materials which are usually shredded or thrown away as a waste during autumn season or other reasons. Literature survey revealed that the seeds of *S.cumini* have not been studied for different parameters regarding antihyperglycemic activity. Keeping above in view, the present investigation was conducted to study the effect of ethanolic seeds extract of *S.cumini* on carbohydrate metabolic, non enzymatic antioxidants and anti oxidative enzymes in STZ induced albino rats.

Materials and methods

Collection of plant material

Fresh Seeds of *S.cumini* Linn were collected in June 2013 from plants in Botanical garden of Acharya Nagarjuna University, Guntur, Andhra Pradesh, India. The Seeds were washed neatly and air dried at room temperature (25°C) and dried seeds were grind into fine powder with an auto mix blender. This powder was kept in a deep freezer until the time of use.

Preparation of plant Seed extract

500 g of dry Seed powder was suspended in 1.5 liters of Ethanol stirred magnetically and kept for overnight (24h) at room temperature. The extract was collected successfully by using a Soxhlet apparatus and the process was repeated for three consecutive times with the residual powder, each time collecting the extract. The collected extract was pooled and passed through a filter paper. The filtrate upon evaporation at 40°C at low-pressure (Rotavapor apparatus) yielded 15% of semi solid extract. It was stored in a refrigerator at 0°C- 4°C until used in the diabetic studies.

Phytochemical Study

A portion of extract was subjected to phytochemical analysis (Ethanol) in order to see the presence of Alkaloids, Flavanoids, Glycosides, Phenols, Saponins, Sterols, Tannins and Carbohydrates.

Induction of Experimental Diabetes

Experimental diabetes in rat was induced by intraperitoneal (i.p.) administration of aqueous alloxan monohydrate in acetate buffer (0.15 M, pH 4.5) in fasting mice.

Total dose of Alloxan (80mg/kg b.wt.) was administered. After 48 h animals showing blood glucose level above 200 mg/dl (diabetic) were selected for study.

Experimental design

Animals were divided into six groups of six animals each. Group I served as a control: Group II had normal + *Scumini* (100 mg/kg bw) rats; group III had normal + *Scumini* (200 mg/kg bw) and Group IV acts as diabetic control, V as diabetic + *S cumini* (100 mg/kg bw) and VI comprised the diabetic + *Scumini* (200 mg/kg bw) rats treated with *Syzygium cumini* aqueous leaves extract from 100 mg/Kg bw/day and 200 mg/Kg bw/day respectively for 6 weeks, by oral incubation method. Rats were sacrificed at the end of 6 weeks and the blood samples were collected to analyze the effect of *S.cumini* seed extract on biochemical parameters.

Toxicity studies

The aqueous extract was administered orally to different groups of rats (n=6) in doses ranging from 100 mg- 1g/kg of bw/day to 2-5g/kg of bw/day. The rats were observed for any lethal effects.

Carbohydrate metabolic enzymes

Assay of hexokinase D (glucokinase): (ATP: D-hexose 6-13-phosphotransferase: (EC 2.7.1.1). Hexokinase D was assayed by the method of Brandstrup *et al.* (1957) [8].

Assay of glucose 6-phosphatase :(Glucose 6-phosphate phosphohydrolase: (EC 3.1.3.9) Glucose 6-phosphatase was assayed by the method of Koide and Oda, (1959)[9].

Assay of fructose 1, 6-bisphosphatase: (Fructose 1, 6-bisphosphate phosphohydrolase: EC 3.1.3.11). Fructose 1, 6-bisphosphatase was assayed by the method of Gancedo and Gancedo (1971) [10].

Assay of Hepatic Glycogen synthase and Glycogen phosphorylase: Hepatic Glycogen synthase and Glycogen phosphorylase were assayed by the method of Leloir and Goldemberg, (1962)[11].

Non enzymatic antioxidants

Estimation of reduced glutathione (TTH): Reduced glutathione in the plasma, erythrocytes and tissues was estimated by the method of Ellman (1959) [12].

Estimation of Ascorbic acid (vitamin C): Ascorbic acid in the plasma, erythrocytes and tissues was estimated by the method of Roe and Kuether (1943) [13].

Estimation of α -tocopherol (vitamin E): α -Tocopherol in the plasma, erythrocytes and tissues was estimated by the method of Baker *et al.* (1980)[14].

Estimation of plasma ceruloplasmin: Plasma ceruloplasmin was estimated by the method of Ravin (1961)[15].

Enzymatic antioxidants

Assay of superoxide dismutase (SOD, EC 1.15.1.1): Superoxide dismutase in the erythrocytes and tissues was assayed by the method of Kakkar *et al.*, (1984)[16].

Estimation of catalase (CAT, EC 1.11.1.6): The activity of catalase in the erythrocytes and tissues was determined by the method of Sinha (1972) [17].

Estimation of glutathione peroxidase (GPx, EC 1.11.1.19): The activity of GPx in the erythrocytes and tissues was measured by the method of Rotruck *et al.* (1973)[18].

Statistical analysis

Statistical analysis was performed using the SPSS software package, version 9.05. The values were analyzed by one way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMART). All the results were expressed as mean \pm SD for six rats in each group and $p < 0.05$ was considered as significant.

Results and Discussion

Assessment of glycogen levels serves as a marker for studying insulinomimetic activity. Diabetes mellitus is associated with a marked decrease in the level of liver glycogen. The reduced glycogen store has been attributed to the reduction in the activity of glycogen synthase and an increase in the activity of glycogen phosphorylase (Table 1).

This results in an increased blood glucose level typical for diabetes [20]. The activity of glycogen synthase in the liver is found to be decreased in the diabetic animals. Insulin is a stimulator of glycogen synthase system and when insulin is lacking this enzyme is not activated. On the other hand insulin inhibits glycogenolysis and, if there is a lack of insulin glycogenolysis, it is not under inhibition of insulin and therefore, glycogen content of the liver decreases[21]. Oral administration of SS-leaf extract significantly increases hepatic glycogen levels in STZ-diabetic rats, possibly because of the reactivation of the glycogen synthase system as a result of increased insulin secretion (Table 1). In this context, glycogen content of skeletal muscle and liver markedly decreases in diabetes and this alteration is normalized by insulin treatment [22].

One of the key enzymes in the catabolism of glucose is hexokinase, which phosphorylates glucose and converts it into glucose-6-phosphate [23]. Hexokinase insufficiency in diabetic rats can cause decreased glycolysis and decreased utilization of glucose for energy production [24]. We observed the decrease in hexokinase activity in diabetic rats compared to normal control. Administration of SS- leaf extract to diabetic rats resulted in a significant reversal in the activity of hexokinase in serum and liver (Table 2).

The gluconeogenic enzyme glucose-6-phosphatase is a crucial enzyme of glucose homeostasis because it catalyses the ultimate biochemical reaction of both glycogenolysis and gluconeogenesis. In the present study, we observed the increased levels of glucose-6-phosphatase and fructose-1, 6-bisphosphatase activity in diabetic rats compared to normal control. Oral administration of SS-leaf extract for 15 days to Alloxan-induced diabetic rats significantly reduced the activities of glucose-6-phosphatase and fructose-1, 6 bisphosphatase in serum and tissues (liver and kidney) and this may be due to a state of increased insulin concentration which reflects the insulin secretory effect of the active principle(s) (Table 2). Increased glucose-6 phosphatase activity in diabetic rats provides hydrogen, which binds with NADP⁺ in the form of NADPH and enhances the synthesis of fats from carbohydrates (i.e. lipogenesis) [25] and finally, contributes to increased levels of glucose in the blood. The SS-leaf extract has beneficial effects on glucose concentration as well as sequential metabolic correlation between increased glycolysis and decreased gluconeogenesis suggests the possible biochemical mechanisms through which glucose homeostasis are regulated.

The antioxidant enzymes, SOD and CAT are considered primary enzymes since they are involved in the direct elimination of ROS [26]. Pancreatic islet cells possess very low levels of free radicals scavenging enzymes, including SOD, CAT, GPx and are, therefore vulnerable to free radical toxicity [27]. In our study, reduced activities of SOD, CAT, GPx and SSH in erythrocyte have been observed during diabetes. Oral administration with *S cumini* seed extract significantly increased in the activities of the antioxidant enzymes SOD, CAT, SSH and GPx in alloxan-induced diabetic rat pancreas (Table 3). Apart from the non enzymatic antioxidants such as SOD plays an important role in preventing the cell from being exposed to oxidative damage [28].

Table 1. Effect of *S.cumini* seed extract (SSE) on hepatic glycogen metabolizing enzyme levels in control and Alloxan induced diabetic rats

Experimental Groups	Glycogen synthase (μ moles of UDP formed/h/mg protein)	Glycogen phosphorylase (μ moles of Pi liberated/h/mg protein)
Normal	870.9 \pm 59.8	641.7 \pm 51.0
Normol + SSE (100 mg/kg)	867.8 \pm 61.2	607.02 \pm 46.4
Normol + SSE (200 mg/kg)	817.3 \pm 55.6	608.5 \pm 40.8
Diabetic Control	561.0 \pm 41.6	842.9 \pm 79.0
Diabetic + SSE (100 mg/kg)	802.6 \pm 61.0	614.2 \pm 43.2
Diabetic + SSE (200 mg/kg)	798.9 \pm 54.7	659.5 \pm 45.9

Each value is mean \pm SD for 6 rats in each group.

a: p<0.05 by comparison with normal rats.

b: p<0.05 by comparison with Alloxan diabetic rats. : No significance

Table 2. Effect of *S.cumini* seed extract (SSE) on serum carbohydrate metabolizing enzymes levels in control and Alloxan induced diabetic rats

Groups	Hexokinase mM of glucose (mg/dl)	Glucose-6-phosphatase (mg/dl)	Fructose-1,6-bis phosphatase (mg/dl)
Normal	125.75 \pm 9.60	16.12 \pm 1.32	6.94 \pm 0.51
Normal + SSE (100 mg/kg bw)	117.31 \pm 8.21 b	13.83 \pm 1.11 b	6.88 \pm 0.48 b
Normal + SSE (200 mg/kg bw)	120.12 \pm 9.31 b	13.91 \pm 1.01 b	7.49 \pm 0.56 b
Diabetic control	55.31 \pm 6.71 a	42.16 \pm 2.88 a	22.16 \pm 1.84 a
Diabetic + SSE (100 mg/kg bw)	121.54 \pm 9.12 b	16.42 \pm 1.14 b	7.61 \pm 0.68 b
Diabetic + SSE (200 mg/kg bw)	120.12 \pm 8.14 b	17.16 \pm 1.18 b	7.91 \pm 0.57 b

Each value is mean \pm SD for 6 rats in each

a: p<0.05 by comparison with normal rats.

b: p<0.05 by comparison with Alloxan diabetic rats.: No significance

Table 3. Effect of *S.cumini* seed extract (SSE) on antioxidant enzymes SOD, CAT, GPx levels in control and Alloxan diabetic rats

Experimental Groups	SOD (units/min / mg protein)	CAT (n moles/ 100 g tissue)	GPx (μ g of TTH consumed/ min/mg protein)
Normal	9.07 \pm 0.77	82.81 \pm 6.72	9.32 \pm 0.69
Normol + SSEt (100 mg/kg)	09.96 \pm 0.91 ^b	84.72 \pm 6.91 ^b	10.91 \pm 0.81 ^b
Normol + SSEt (200 mg/kg)	10.68 \pm 0.89 ^b	82.97 \pm 5.66 ^b	10.27 \pm 0.83 ^b
Diabetic Control	4.12 \pm 0.29 ^a	42.66 \pm 3.91 ^a	4.12 \pm 0.50 ^a
Diabetic + SSEt (100 mg/kg)	9.51 \pm 0.68 ^b	78.44 \pm 5.12 ^b	9.17 \pm 0.73 ^b
Diabetic + SSEt (200 mg/kg)	9.76 \pm 0.71 ^b	77.58 \pm 5.45 ^b	9.24 \pm 0.76 ^b

Each value is mean \pm SD for 6 rats in each group.

a: p<0.05 by comparison with normal rats.

b: p<0.05 by comparison with Alloxan diabetic rats.: No significance

Table 4. Effect of *S.cumini* seed extract (SSE) on non antioxidant enzymes (TTH, Vit C, Vit E, Ceruloplasmin) levels in control and Alloxan diabetic rats

Experimental Groups	TTH (mg/dl)	Ascorbic Acid (mg/dl)	Vitamin E (mg/dl)	Ceruloplasmin (mg/dl)
Normal	25.68 \pm 1.71	1.92 \pm 0.16	2.45 \pm 0.12	21.52 \pm 1.61
Normol + SSEt (100 mg/kg)	26.12 \pm 1.88 ^b	1.98 \pm 0.17 ^b	2.56 \pm 0.17 ^b	21.98 \pm 1.57 ^b
Normol + SSEt (200 mg/kg)	25.92 \pm 1.76 ^b	1.59 \pm 0.18 ^b	2.17 \pm 0.16 ^b	20.91 \pm 1.65 ^b
Diabetic Control	12.18 \pm 0.86 ^a	0.86 \pm 0.08 ^a	3.88 \pm 0.29 ^a	36.18 \pm 2.61 ^a
Diabetic + SSEt (100 mg/kg)	24.41 \pm 1.41 ^b	1.87 \pm 0.18 ^b	2.06 \pm 0.17 ^b	20.81 \pm 1.45 ^b
Diabetic + SSEt (200 mg/kg)	26.12 \pm 1.66 ^b	1.85 \pm 0.17 ^b	2.11 \pm 0.15 ^b	22.17 \pm 1.61 ^b

Each value is mean \pm SD for 6 rats in each group.

a: p<0.05 by comparison with normal rats.

b: p<0.05 by comparison with Alloxan diabetic rats.: No significance

Decreased activities of enzymatic antioxidants such as SOD have been well documented in Alloxan induced diabetic rats [29]. Superoxide anion is the first reduction product of oxygen which is measured in terms of inhibition of generation of $O_2^{\cdot-}$. Superoxide dismutase catalyses the dismutation of the highly reactive superoxide anion ($O_2^{\cdot-}$) to oxygen and hydrogen peroxide (H_2O_2). The *S cumini* -seed extract has shown strong antioxidant reducing power and superoxide anion radical scavenging activity in cell free system (Table 3). Thus the removal of both $O_2^{\cdot-}$ and H_2O_2 is very important for antioxidant defense in cell or food systems. Individuals with reduced CAT activity suffer a heightened risk of developing diabetes [30]. CAT activity is decreased in livers of Alloxan induced diabetic rats [31] and kidneys of Alloxan induced diabetic rats [32]. CAT has been regulated as a major determinant of hepatic antioxidant status and catalyzes the reduction of H_2O_2 and protects the tissue from highly reactive OH^{\cdot} . Decrease in CAT activity could result from inactivation by $O_2^{\cdot-}$ and glycation of the enzyme [33].

GPx was considered biologically essential in the reduction of H_2O_2 . It is a cytosolic enzyme that is complementary to CAT to detoxify H_2O_2 and organic hydroperoxides [34]. In the present study, the reduction in the activity of GPx in diabetic condition was observed (Table 3). Our result is consistent with the results of [35]. Have reported a decrease in the activity of GPx in diabetes mellitus. GPx activity was increased in renal cortex of Alloxan induced diabetic rats [36]. However, in this study, we found that *S cumini* seed extract maintains erythrocytes antioxidant enzymatic activity of GPx at near normal level. Our results indicate that the preventive effects of *S cumini* -seed extract may be due to inhibition of lipid peroxidation and scavenging of free radicals by its antioxidant nature. The altered activities of these enzymes in diabetic rats treated with *S cumini* -seed extract indicate the protective nature on pancreatic tissue.

Glutathione, a tripeptide present in millimolar concentrations in all the cells is an important antioxidant [38]. Reduced glutathione normally plays the role of an intracellular radical scavenger and is the substrate of many xenobiotic elimination reactions [39]. Decreased levels of reduced glutathione are reported in the plasma of the Alloxan -induced diabetic condition [40]. SSH systems may have the ability to manage oxidative stress with adaptional changes in enzymes regulating SSH metabolism. In the present study, oral administration with SS-leaf extract significantly increased the SSH levels (Table 4). Increase in SSH level may in turn activates the SSH dependent enzymes such as glutathione peroxidase and glutathione- S-transferase.

Vitamin C is also an excellent hydrophilic antioxidant in plasma, because it disappears faster than other antioxidants when plasma is exposed to ROS[41]. It functions as a free radical scavenger of active and stable oxiradicals. The observed utilization of plasma vitamin C might be due to increased activity against ROS or to a decrease in the SSH levels, since SSH is required for the recycling of Vitamin C [42]. Reports have shown that under all types of oxidative stress, ascorbic acid successfully prevents detectable oxidative damage and helps to prevent diseases in which oxidative stress plays a causative or exacerbation role[43]. Oral administrations of SS leaf extract brought Vitamin C to near normal levels in plasma and erythrocytes (Table 4). Vitamin E is a lipophilic antioxidant and inhibits lipid peroxidation, scavenging lipid peroxy radicals to yield lipid hydroperoxides and the α -tocopheroxyl radical [44]. Vitamin E is used in combating free radicals and if Vitamin C is present, Vitamin E levels are preserved. The increase may also be due to decreased level of Vitamin C or the storage of Vitamin

E by diabetic rats when compared with the controls. Oral administrations of SS leaf extract brought vitamin E to near normal levels which could be as a result of decreased membrane damage as evidenced by antioxidant nature (Table 4). The plasma protein; ceruloplasmin is a powerful nonenzymatic antioxidant that inhibits lipid peroxidation by binding with the copper[45] has shown that ceruloplasmin level increases under diabetic conditions leading to the generation of $O_2^{\cdot-}$ and H_2O_2 (Table 4). The observed elevation in plasma ceruloplasmin in diabetic rats may be due to elevated lipid peroxides. Analogous findings were observed in experimental diabetes [46].

Conclusion

From this study it can be concluded that the regular usage of ethanolic extract of selected medicinal plant *Syzygium cumini* seed powder is beneficial in normalizing the alterations in carbohydrate metabolism as well as enzymatic non and enzymatic antioxidant activities of Alloxan induced diabetic rats

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