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Effect of *Syzigium cumini* in Plasma Antioxidants on Alloxan-Induced Diabetes in Rats

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Summary We undertook the present study to evaluate the antioxidant activity of an aqueous extract of the seeds of *Syzigium cumini*, an indigenous plant present in different parts of India, South-East Asia and Eastern Africa, toward experimental diabetes. Administration of the extract for 6 weeks resulted in significant reductions in plasma lipid peroxide, ceruloplasmin and α-tocopherol and a significant elevation in plasma reduced glutathione and vitamin C in alloxan diabetic rats. Insulin restored all the parameters to their normal values. The seed extract was also more effective than glibenclamide in restoring the values of these parameters.

Key Words: *Syzigium cumini*, alloxan diabetes, antioxidants, aqueous extract

Active oxygen metabolism plays a role in the normal functioning of the β-cells of islets of Langerhans. Many studies carried out in recent years have shown elevated lipid peroxidation in diabetes. Increased circulating lipid peroxides have been reported in diabetic patients [1] and experimental diabetic animals [2]. The level of peroxides in tissues were elevated in diabetic rats [3]. Circulating lipid peroxides were shown to be capable of initiating atherosclerosis [4], which is a well-known late feature of diabetes. It was also shown that rabbits injected with linoleic acid hydroperoxides developed aortic lesions [5].

The level of lipid peroxidation in cells is controlled by various cellular defense mechanisms consisting of enzymatic and nonenzymatic scavenger systems [6, 7]. The levels of these defense mechanism are altered in diabetes [8]; and, therefore, the ineffective scavenging of free radicals plays a crucial role in determining tissue injury.

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Syzigium cumini Linn. (family: Myrtaceae), locally known as Jamun, is native to India, thrives easily in a tropical climate and is found in many parts of our subcontinent. It is a large evergreen tree that grows widely in the Indogangetic plains and also in the Cauvery delta of Tamil Nadu. Besides India, it also occurs in South-East Asia and Eastern Africa [9]. Jamun is widely cultivated in India for its delicious fruits.

The seeds have been considered as an indigenous source of medicines to have hypoglycemic [10], antipyretic [11], antiinflammatory [12] and neuropsychopharmacological [13] actions. In an earlier study, we reported the hypolipidemic action of Jamun seeds [14]. No previous study has been done concerning their action on the plasma antioxidant status in experimental diabetes.

Presently we report the effect of Jamun seed extract (JSEt) at different doses on plasma lipid peroxide and nonenzymatic antioxidants in alloxan diabetic rats.

MATERIALS AND METHODS

Animals. Male albino rats of the Wistar strain and in the weight range of 130–160 g were procured from the Central Animal House, Rajah Muthiah Medical College, Annamalai University. They were housed (6/cages) in plastic cages ($47 \times 34 \times 18$ cm) lined with husk renewed every 24 h and had free access to drinking water and food.

Plant material. Jamun seeds were collected during June 1996 from rural areas around Nagercoil, Kanyakumari District, in the province of Tamil Nadu, India. The seeds were authenticated with the help of a botanist at the Annamalai University.

Preparation of aqueous Jamun seed extract (JSEt). The Jamun seed was dried and powdered, and a suspension of 100 g in 200 ml distilled water was stirred magnetically overnight at room temperature. This was repeated three consecutive times. The extract was evaporated to dryness under reduced pressure in a rotary evaporator. The residual extract was dissolved in saline and used in the study.

Experimental induction of diabetes. Diabetes in rats was induced by intraperitoneal injection of 150 mg/kg of alloxan monohydrate [15] (Sigma Chemical Co., St. Louis, MO). The mortality rate after alloxan treatment was 45%. After 2 weeks rats with blood glucose in the range of 200–260 mg/100 ml were taken for the study.

Experimental design. Animals were divided into 7 groups, each containing 12 rats. Group 1 control rats given 0.5 ml of saline; Group 2 diabetic rats given 0.5 ml of saline; Group 3 diabetic rats given JSEt (2.5 g/kg body weight) in saline solution daily for 6 weeks; Group 4 diabetic rats received JSEt (5.0 g/kg body weight) daily for 6 weeks; Group 5 diabetic rats received JSEt (7.5 g/kg body weight) daily for 6 weeks; Group 6 diabetic rats received glibenclamide orally in saline solution (600 μg/kg body weight) daily for 6 weeks; Group 7 diabetic rats received protamine zinc insulin (6 units/kg body weight) daily for 6 weeks.

After 42 days of treatment, blood was collected in heparinized tubes, and the plasma was separated by centrifugation. Plasma lipid peroxide was measured by the method of Yagi [16]. Ceruloplasmin [17], α-tocopherol [18], reduced glutathione (GSH) [19] and vitamin C [20] were determined on the same day.

Statistical analysis. Statistical analysis was done by Student's t-test. Results were expressed as the means$\pm$SD from 12 rats in each group. p-Values of <0.001 were considered significant.

RESULTS

Table 1 shows the levels of plasma lipid peroxide in normal and experimental rats. The plasma lipid peroxide were significantly higher in the diabetic rats than in the normal ones ($p<0.001$). Rats administered JSEt (2.5 and 5.0 g/kg body weight) or glibenclamide showed significantly lower plasma lipid peroxide levels as compared with diabetic rats ($p<0.001$)

Table 2 shows the plasma ceruloplasmin and α-tocopherol levels in normal and experimental rats. Both were significantly higher in diabetic rats as compared

Table 1. Effect of JSEt on plasma lipid peroxide levels in diabetic rats (mean$\pm$SD from 12 rats in each group).

Group	Lipid peroxide (nmol/ml)
Normal	1.2$\pm$0.026
Diabetic	2.1$\pm$0.021*
Diabetic+JSEt (2.5 g/kg)	1.5$\pm$0.014**
Diabetic+JSEt (5.0 g/kg)	1.4$\pm$0.016**
Diabetic+JSEt (7.5 g/kg)	2.1$\pm$0.018$^{n.s}$
Diabetic+glibenclamide	1.6$\pm$0.015**
Diabetic+insulin	1.3$\pm$0.018**

*As compared with normal, $p<0.001$. **As compared with diabetic, $p<0.001$. $^{n.s}$Not significant.

Table 2. Effect of JSEt on plasma ceruloplasmin and α-tocopherol in diabetic rats (mean$\pm$SD from 12 rats in each group).

Group	Ceruloplasmin (mg/dl)	α-Tocopherol (mg/dl)
Normal	15.98$\pm$6.4	1.63$\pm$0.21
Diabetic	29.40$\pm$3.7*	2.78$\pm$0.30*
Diabetic+JSEt (2.5 g/kg)	19.21$\pm$5.8**	2.26$\pm$0.29**
Diabetic+JSEt (5.0 g/kg)	18.50$\pm$6.2**	2.12$\pm$0.40**
Diabetic+JSEt (7.5 g/kg)	28.90$\pm$5.2$^{n.s}$	2.71$\pm$0.34$^{n.s}$
Diabetic+glibenclamide	19.73$\pm$3.2**	2.31$\pm$0.19**
Diabetic+insulin	16.92$\pm$3.8**	1.86$\pm$0.23**

*As compared with normal, $p<0.001$. **As compared with diabetic, $p<0.001$. $^{n.s}$Not significant.

Table 3. Effect of JSEt on plasma reduced glutathione and vitamin C in diabetic rats (mean±SD from 12 rats in each group).

Group	Reduced glutathione (mg/dl)	Vitamin C (mg/dl)
Normal	26.94±2.1	1.98±0.31
Diabetic	16.90±1.9*	1.20±0.24*
Diabetic+JSEt (2.5 g/kg)	24.83±3.1**	1.60±0.22**
Diabetic+JSEt (5.0 g/kg)	25.12±3.6**	1.62±0.26**
Diabetic+JSEt (7.5 g/kg)	17.20±1.6$^{n.s}$	1.23±0.32$^{n.s}$
Diabetic+glibenclamide	24.67±2.2**	1.58±0.21**
Diabetic+insulin	27.12±2.9**	1.76±0.20**

*As compared with normal, $p<0.001$. **As compared with diabetic, $p<0.001$. $^{n.s}$Not significant.

with their values in normal rats ($p<0.001$). Administration of JSEt (2.5 and 5.0 g/kg body weight) or glibenclamide decreased significantly the ceruloplasmin and α-tocopherol levels as compared with those in diabetic rats ($p<0.001$).

Table 3 shows that plasma GSH and vitamin C levels were significantly lower in diabetic rats than in normal rats ($p<0.001$). Administration of JSEt (2.5 and 5.0 g/kg body weight) or glibenclamide increased significantly the GSH and vitamin C levels as compared with their levels in diabetic rats ($p<0.001$).

For all the parameters studied, JSEt at doses of 2.5 and 5.0 g/kg body weight showed significant antioxidant action. Administration of JSEt at higher doses (7.5 g/kg body weight) to alloxan diabetic rats did not have a significant effect. The effect exerted by JSEt was more effective than that by glibenclamide. Insulin brought back all the parameters to near normal.

DISCUSSION

Oral administration of an aqueous extract of Jamun seed has shown antioxidant effects against alloxan diabetic rats. JSEt at doses of 2.5 and 5.0 g/kg body weight lowered significantly the plasma lipid peroxide, ceruloplasmin, and α-tocopherol levels and significantly increased the plasma GSH and vitamin C levels.

The level of plasma lipid peroxide is raised in alloxan diabetes. An increase in the level of lipid peroxides in plasma is generally thought to be the consequences of increased production of and liberation into the circulation of tissue lipid peroxides due to pathological changes [21]. In this context Selvam and Anuradha reported an increase in lipid peroxides in the plasma of alloxan diabetic rats [21].

Changes in the antioxidants are observed in diabetic conditions. The plasma protein ceruloplasmin is a powerful free radical scavenger that inhibits lipid peroxidation by binding to copper [22]. Ceruloplasmin levels increase under conditions leading to the generation of oxygen products such as the superoxide radical and hydrogen peroxide [23]. The observed rise in plasma ceruloplasmin in

diabetic rats may be due to increased lipid peroxides. In fact, Suresh Kumar and Menon [24] reported increased serum ceruloplasmin in alloxan diabetic rats.

Glutathione is an important inhibitor of free radical-mediated lipid peroxidation [25]. We observed lower levels of plasma glutathione in alloxan diabetic rats. It appears that generation of oxygen radicals by increased levels of glucose causes utilization of GSH and thus lowers GSH levels in the plasma in diabetes. In this context other workers have reported diminished levels of blood GSH in alloxan diabetes [21].

Vitamin C is an excellent hydrophilic antioxidant in plasma, because it disappears faster than other antioxidants when plasma is exposed to reactive oxygen species [26]. We observed a decreased level of plasma vitamin C in the diabetic rats. This decrease could have been due to increased utilization of vitamin C as an antioxidant defense against increased reactive oxygen species or to a decrease in the GSH level, since GSH is required for the recycling of vitamin C [27]. Selvam and Anuradha [21] also reported lowered plasma vitamin C in alloxan induced diabetes.

The most important lipid-soluble, radical-scavenging antioxidant is α-tocopherol. It interrupts the chain reaction of lipid peroxidation by reacting with lipid peroxy radicals, thus protecting the cell structures against damage [28]. The elevated level of α-tocopherol found in the diabetic rats is compatible with the hypothesis that α-tocopherol excess in the plasma of diabetics plays a protective role against increased peroxidation and hence against increased platelet aggregability [21]. Increased levels of plasma α-tocopherol have been reported by other workers in alloxan diabetic rats [24].

Our findings showed that oral administration of JSEt to alloxan diabetic rats for 6 weeks decreased the plasma lipid peroxide, ceruloplasmin, and α-tocopherol levels and increased plasma GSH and vitamin C levels. Although we tested three levels of JSEt, 2.5, 5.0, and 7.5 g/kg body weight, the antioxidant effect was only detectable with the 2.5 and 5.0 g doses. At the 7.5 g level there was no significant alteration. It is not clear as to why there was no significant effect at this highest dose. In this context we have shown that an aqueous extract of Jamun seeds at high doses did not have a significant hypolipidemic effect in alloxan diabetic rats [14]. JSEt has an antioxidant property in experimental diabetes.

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