Introduction

It is believed that, oxidative stress due to chronic hyperglycaemia plays an important role in the etiology of diabetic complications. Many detrimental effects in diabetes are linked to the increased serum glucose that leads to an elevated level of reactive oxygen species (ROS) (Liu & Gutterman, 2002). It is unclear which major chemical path way is responsible for enhanced ROS production in diabetic micro vessels. However, several endogenous systems have been proposed as possible source for excess ROS in diabetes which include inhibition of Xanthine dehydrogenase (Halliwell & Gutterudge, 1999), glycation of Cu, Zn, SOD (Williamson et al, 1993), generation of advanced glycosylated end products (Lyons,1993), and increased production of diacylglycerol with glycolytic intermediates (Ishi et al, 1998). Thus, excess generation of these ROS further causes oxidative stress which leads to pathophysiology of several human diseases (Maxwell, 1995). Endogenous antioxidant enzymes, such as SOD, CAT, GSH and GPx have been demonstrated to protect against oxidative stress (Peng & Li, 2002). There has been increasing scientific interest on effect of induced endogenous antioxidants and /or exogenous antioxidants and chemicals in the prevention of adverse consequences of oxidative stress (Peng & Li, 2002; Otieno et al, 2000; Cuzzocrea et al, 2001; Kwak et al, 2001). However, it is not feasible to use exogenous antioxidant chemicals for the protection of oxidative stress and they have limited cell permeability, short half life in vivo (Dhalla et al, 2000), and toxic properties (Satoh et al, 1999). On the other hand, plant drugs and extracts are frequently considered to be less toxic and more free from side effects, so attempt have been made to utilize herbal products to control diabetes mediated oxidative stress (Prince et al, 1998; Tahiliani & Kar, 1999; Chaurasia et al, 2000; Muruganandan et al, 2001). Many plants have been

Protective effect of *Syzygium cuminii* (Linn.) Skeels seed extract on lipid peroxidation in alloxan induced diabetic rats

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Abstract

The effect of Black plum, *Syzygium cuminii* (Linn.) Skeels (syn. *S. jambolanum* DC.) seed extract on lipid peroxidation in alloxan induced diabetic rats was studied. Alloxan 150mg (mg/kg body weight) increased significantly the glucose level in blood and induced the Lipid Peroxidation (LPO) in liver. The antioxidant enzymes Catalase (CAT), Reduced Glutathione (GSH), Glutathione peroxidase (GPx), Superoxide dismutase (SOD) in liver were decreased. Oral administration of the extract for 15 days to alloxan treated animals showed remarkable increase in the level of antioxidant enzymes and reduced the level of lipid peroxidation activity and blood glucose. The results suggest that seed extract of the plant is an antioxidant and acute hyperglycaemic drug and might be used in the regulation of lipid peroxidation without detectable adverse side effects.

Keywords : Black plum, *Jamun, Syzygium cuminii, Syzygium jambolanum*, Seed extract, Antioxidant, Alloxan, Diabetes, Lipid peroxidation, Oxidative stress.

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Research Article

Black plum fruits and seeds

of diacylglycerol with glycolytic intermediates (Ishi et al, 1998). Thus, excess generation of these ROS further causes oxidative stress which leads to pathophysiology of several human diseases (Maxwell, 1995). Endogenous antioxidant enzymes, such as SOD, CAT, GSH and GPx have been demonstrated to protect against oxidative stress by eliminating ROS (Peng & Li, 2002). There has been increasing scientific interest on effect of induced endogenous antioxidants and /or exogenous antioxidants and chemicals in the prevention of adverse consequences of oxidative stress (Peng & Li, 2002; Otieno et al, 2000; Cuzzocrea et al, 2001; Kwak et al, 2001). However, it is not feasible to use exogenous antioxidant chemicals for the protection of oxidative stress and they have limited cell permeability, short half life in vivo (Dhalla et al, 2000), and toxic properties (Satoh et al, 1999). On the other hand, plant drugs and extracts are frequently considered to be less toxic and more free from side effects, so attempt have been made to utilize herbal products to control diabetes mediated oxidative stress (Prince et al, 1998; Tahiliani & Kar, 1999; Chaurasia et al, 2000; Muruganandan et al, 2001). Many plants have been
shown to have antioxidant and antidiabetic activity and successfully used as ROS scavenging agents (Marles & Farnsworth, 1995; Lee & Shibamoto, 2001).

**Syzygium cuminii** (Linn.) Skeels syn. *S. jambolanum* DC, *Eugenia jambolana* Lam., commonly known as Black plum (Hindi–Jamun), found throughout India is well-known for its edible fruits and medicinal value. The leaves are used to reduce the blood glucose level (diabetes) in traditional practices (Shanker Gopal Joshi, 2000) and possess antibacterial and fungicidal activities also (Chandrasekaran & Venkatesalu, 2004). The fruits and seeds are reported to be used for the treatment of diabetes (With India –Raw Materials, 1976). In the present study an attempt has been made to scrutinize the level of antioxidant enzymes in response to alloxan and seed extract of *S. cuminii* on rats.

**Materials and Methods**

**Collection of seeds**

Ripe fruits were collected from the tree and washed well to remove the pulp and collect the seeds.

**Preparation of extract**

Seeds were shade dried, powdered and exhaustively extracted at 60°C with 95% ethanol using Soxhlet apparatus (Suffness & Douros, 1979). The solvent was evaporated under reduced pressure to obtain the crude extract. The residue was weighed and dissolved in carrier oil (coconut oil) to get required concentration.

**Experimental design**

Male albino rats of Wistar strain weighing 100-130g were used for the present study. The animals were maintained under constant environmental condition in the laboratory and they were fed with commercial pellet feed and given water *ad libitum*. The rats were divided into 4 groups of 6 animals each. Group I served as control, Group II rats were made diabetic by intraperitonial injection of 150 mg/kg body weight of alloxan in saline (0.9% NaCl). Group III alloxan induced diabetic rats received 1.0ml of seed extract and Group IV rats (non-diabetic) received only saline and seed extract.

The experimental rats were sacrificed after 15 days of treatment with the seed extract. The liver was dissected out and immediately placed in ice, weighed and 10% homogenate was prepared in ice chilled 10% KCl solution. After centrifugation at 2000 rpm for 10 minutes the clear supernatant was used to measure the antioxidant enzymes level. SOD measurement was done based on the ability of the enzyme to inhibit oxygen dependent auto-oxidation (Fridovich, 1976), CAT was estimated based on the ability of CAT to oxidize hydrogen peroxide according to the method of Beers and Sizer (1952), reduced GSH was assayed by the method of Moron *et al* (1979), GPx was measured by the method of Rotruck *et al* (1973) using H$_2$O$_2$ as substrate. Lipid peroxidation was estimated by thiobarbituric acid reaction as described by Okhawa *et al* (1979). The blood glucose level was measured colorimetrically using diagnostic kit manufactured by Span Diagnostic Private Ltd, Surat, India.

**Statistical analysis**

The experimental data were analyzed by the statistical method of standard deviation and students “t” test.

**Results**

The level of antioxidant enzymes: CAT, reduced GSH, GPx and SOD, Lipid peroxidation and blood glucose are given in Table 1. The antioxidant enzyme activities significantly decreased in liver, but the blood glucose and lipid peroxidation level were increased in alloxan induced diabetic rats. When treated with this seed extract the alloxan induced diabetic rats showed increase in antioxidant activities than the untreated rats with extract. The lipid peroxidation and blood glucose level were reduced in the seed extract treated rats. In *S. cuminii* seed extract administrated control rats, the lipid peroxidation enzyme levels were near normal values similar to untreated control rats.

**Discussion**

There was a significant increase in lipid peroxidation while the specific activity of CAT, reduced GSH, GPx and SOD were decreased in alloxan induced rats as compared with corresponding control group. This may be due to the adverse effect of alloxan which caused massive reduction in insulin release by way of destructing β-cells of islets of langerhans and leading to hyperglycaemia (Goldner & Gomori, 1943). Hyperglycaemia increases the production of ROS (Liu & Gutterman, 2002) and it may increase the...
lipid peroxidation in alloxan treated test animals. In addition, it is probable that excess ROS may alter superoxide dismutase and catalase in the presence of alloxan (Yadav et al, 1997). Oxidative tissue damage triggered by D-galactosamine is believed to be due to the formation of reactive hydroxyl radicals which are the initiation of lipid peroxidation chain reaction (Lin et al, 1995; Gene et al, 1998).

Black plum seed extract treated diabetic rats showed a significant increase in the level of CAT, reduced GSH, GPx and SOD in the liver. It may be due to the singlet oxygen quenching ability of phenolic compounds present in the seed extract. The antioxidant properties of flavonoids from different plant sources have been reported by earlier workers (Husain et al, 1987; Robak & Gryglewski, 1988; Rios et al, 1992; Calomme et al, 1995). Plant extract treatment increased the activity of SOD and CAT and it scavenges superoxide radicals and reduces cellular damage caused by free radicals (Prince et al,1998; Chaurasia et al, 2000; Lee & Shibamoto, 2001). From our results, it is evident that induced SOD serves to remove O$_2^-$ by accelerating the formation of H$_2$O$_2$. Since, H$_2$O$_2$ is harmful to cells, CAT and GSH, GPx were increased in black plum treated rats. The increased level of CAT and GSH, GPx would help to metabolise H$_2$O$_2$ to water. GSH reductase helps GSH, GPx by way of acting as GSH reducing agent and also increased to protect the cells from pro-oxidant. Lipid peroxidation was significantly decreased in the extract treated group of the present study. The observed reduction in the level of lipid peroxidation may be due to the presence of antioxidant compounds such as β-carotene and eugenol in S. cuminii. It has been reported that

Table 1: Levels of Blood glucose, LPO, GPx, GSH, SOD and CAT in liver of control and experimental animals

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (Group I)</th>
<th>Alloxan 150 mg/kg body weight (Group II)</th>
<th>Alloxan induced Diabetic + seed extract (Group III)</th>
<th>Control + seed extract (Group IV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipid peroxidation (LPO)</td>
<td>26.4 ±0.45</td>
<td>40.0±0.4</td>
<td>31.34±0.5</td>
<td>26.0±0.21</td>
</tr>
<tr>
<td>Reduced Glutathione (GSH)</td>
<td>11.6±0.4</td>
<td>8.67±0.3***</td>
<td>13.21±0.63</td>
<td>12.2±0.5</td>
</tr>
<tr>
<td>Glutathione Peroxidase (GPx)</td>
<td>636.2±13.94</td>
<td>575.1± 14.0**</td>
<td>650.73±15.8</td>
<td>638.1±12.67</td>
</tr>
<tr>
<td>Superoxide dismutase (SOD)</td>
<td>80.05±0.6</td>
<td>63.6±0.60***</td>
<td>82.2±0.5</td>
<td>80.02±0.51</td>
</tr>
<tr>
<td>Catalase (CAT)</td>
<td>69.40±0.6</td>
<td>64.6±0.45</td>
<td>71.5±0.51</td>
<td>69.00±0.60</td>
</tr>
<tr>
<td>Blood glucose</td>
<td>270.0±5.8</td>
<td>335.0±4.9</td>
<td>281.20±3.0</td>
<td>270.0±4.0</td>
</tr>
</tbody>
</table>

**P < 0.01;  ***P <0.001  Values are mean ± S.D.
Units : SOD- Units/mg protein; CAT - n moles of H$_2$O$_2$ decomposed/min/mg protein; GPx - n moles of GSH oxidized/min/mg protein; LPO- n moles of malondialdehyde/mg protein; GSH-moles /mg protein.Blood Glucose mg/100ml
eugenol plays an important role in the production of lipid peroxidation (Lee & Shibamoto, 2001) by scavenging superoxide anion radicals. Decrease in lipid peroxides could also be due to the reduction of free fatty acids and increased level of free radicals scavenging enzymes (Chithra & Leelamma, 1999). Alcoholic extract of Hypericum perforatum Linn. shoot inhibited lipid peroxidation partly by scavenging the OH radicals and chelating of Fe$^{2+}$/Fe$^{3+}$ iron and thereby inhibiting the production of super oxide anions (Tripathi & Pandey, 1999). Similarly, ethyl acetate and methanol extracts of Pleurotus florida showed significant lipid peroxidation inhibition activity (Nayana Jose & Janardhanan, 2000). In the present investigation the inhibition of lipid peroxidation and enhancement of the antioxidant enzymes level could be due to the alcoholic extract of S. cuminii seeds which possess a strong free radical scavenging properties.

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References


