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Antidiabetic and Antioxidant Effect of *Semecarpus anacardium* Linn. Nut Milk Extract in a High-Fat Diet STZ-Induced Type 2 Diabetic Rat Model

Haseena Banu Hedayaathullah Khan, Kaladevi Siddhi Vinayagam, Ashwini Sekar, Shanthi Palanivelu, & Sachdanandam Panchanadham

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**ABSTRACT.** *Semecarpus anacardium* commonly known as marking nut has been used in the Siddha system of medicine against various ailments. The antidiabetic and antioxidant potential of the drug was evaluated in Type 2 diabetic rats induced by feeding a high-fat diet (HFD) for 2 weeks followed by single intraperitoneal injection of streptozotocin (STZ) 35 mg/kg body weight. Three days after STZ induction, the hyperglycemic rats were treated with *Semecarpus anacardium* nut milk extract (SA) orally at a dosage of 200 mg/kg body weight daily for 30 days. Metformin (500 mg/kg body weight, orally) was used as a reference drug. The fasting blood glucose, insulin, Hb, HbA1c levels, and HOMA-IR and HOMA-β were measured, and also the levels of lipid peroxidation and antioxidant enzymes were observed. SA significantly (*p* < .05) reduced and normalized blood glucose levels and also decreased the levels of HbA1c as compared with that of HFD STZ control group. SA treatment also significantly (*p* < .05) increased the levels of antioxidant enzymes while decreasing the levels of lipid peroxidation. The potential antihyperglycemic action and antioxidant role might be due to the presence of flavonoids in the drug.

**KEYWORDS.** Diabetes mellitus, antioxidants, HbA1c, HOMA-IR, streptozotocin

**INTRODUCTION**

Type 2 diabetes mellitus (T2DM) is the most common endocrine and metabolic disorder, affecting more than 170 million people worldwide, and is predicted to affect over 365 million people by the year 2030 (Wild, Roglic, Green, Sicree, & King, 2004). Both insulin resistance and insulin deficiency are characteristic features of T2DM (Srinivasan, Viswanad, Asrat, Kaul, & Ramarao, 2005).

Oxidative stress is the major cause and consequence of T2DM. In hyperglycemia, autoxidation of glucose increases the formation of free radicals beyond the capacity of the defense system to neutralize it and cause oxidative stress (Kaneto et al., 2005). It is known to impair both the biosynthesis of insulin in pancreas and...
damage the insulin receptors, thereby decreasing the number of sites available for the binding of insulin (Kaneto et al., 2005). Thus, antioxidant therapy in diabetes may be helpful in relieving symptoms and complications observed in diabetes. Herbal products or plant products are rich in phenolic compounds and flavonoids and other constituents, which show reduction in blood glucose levels (Ji, Li, & Zhang, 2009). Flavonoids are a group of naturally occurring polyphenolic compounds present in plants. Although the antidiabetic properties of flavonoids can be explained by a wide variety of mechanisms (Kato et al., 2008), the antioxidant capacity of this group of natural compounds plays an important role in the prevention of hyperglycemia and related complications (Vitor et al., 2004). Several traditional medicinal plants, such as *Averrhoa bilimbi* (Tan, Tan, & Pushparaj, 2005), *Potentilla discolor* Bunge (Li et al., 2010), and *Butea monosperma* (Kehkashan & Siddiqui, 2011), that possess a substantial quantity of antioxidant components have been found to be useful against diabetes and its related complications. Hence, there is a huge prospect of development of potential hypoglycemic agents coupled with antioxidant activity from traditional medicinal plants to combat diabetes and its complications (Vosough-Ghanbari et al., 2008).

*Semecarpus anacardium* Linn. is a moderate-sized deciduous tree, reaching a height of up to 12–15 m, with rough dark brown bark. Commonly distributed in the sub-Himalayan regions of India, in Malaysia, Myanmar, Singapore, China, Northern Australia, and Africa, several phenolics including biflavonoids have been found to be present in the nut as well as in the oil of *Semecarpus anacardium* (Rastogi & Mehrotra, 1980). The chief constituents present in the nut milk extract include trihydroxyflavone, semecarpol, anacardoside, and bhilawanols (Sujatha & Sachdanandam, 2002; Surveswaran, Cai, & Corke, 2007). Many pharmacological properties such as antitumor, antimicrobial, anti-inflammatory, and antirheumatic have been attributed to the nut extracts (Patwardan, Ghoo, & David, 1988; Nair et al., 2009). Several studies have also established the anticancer potency of *Semecarpus anacardium* nut milk extract (SA; Premalatha, Muthulakshmi, & Sachdanandam, 1999), by its immunomodulatory potency and by the prevention of enhanced lipid peroxidation (LPO; Premalatha & Sachdanandam, 2000). The alkaloids isolated from *Semecarpus anacardium* nuts oil have been known to induce apoptosis in human tumor cells of diverse origin (Chakraborty, Roy, Taraphdar, & Bhattacharya, 2004). Toxicity studies of the drug have shown that the drug is safe and does not induce any toxic manipulation on the biochemical parameters investigated in rats (Vijayalakshmi, Muthulakshmi, & Sachdanandam, 2000).

The present investigation was carried out to study the effect of SA on marker enzymes and antioxidant enzymes in a high-fat diet (HFD) STZ-induced Type 2 diabetic rat model. The effect of SA was compared with conventional antidiabetic agent, metformin (MET), which belongs to the class known as the biguanides that act by controlling hepatic glucose production (Frendell, Glazer, & Zhan, 2003).

**MATERIALS AND METHODS**

**Animals and Diet**

Male Sprague Dawley rats (230 ± 20 g) were used in this study. The animals were purchased from Central Animal House, Institute of Basic Medical Sciences,
Chennai, India. The animals were housed in large spacious cages, bedded with husk, and were given food and water ad libitum. The animal room was well ventilated with a 12-h light/dark cycle, throughout the experimental period. Animal experimentation was conducted according to the current institutional regulations (IAEC No.: 02/081/07). The animals were maintained on a commercial rat feed manufactured by M/s. Pranav Agro Industries Ltd., India, under the trade name “Amrut” rat/mice feed.

**Drugs and Chemicals**

SA was prepared according to the *Formulary of Siddha Medicine* (Narayanaswami & Uthamaramayan, 1972). Streptozotocin (STZ) was obtained from Sigma Chemicals (St. Louis, MO, USA). All other chemicals and solvents used were of analytical grade.

**Experimental Design**

The animals were divided into 5 groups of 6 animals each. Rats were fed with an HFD that consisted of 10% lard, 20% sucrose, 2.5% cholesterol, and 1.0% cholate for 2 weeks (Xie & Du, 2005). After 2 weeks, the animals were kept in an overnight fast and injected with STZ (35 mg/kg body weight in 0.1 M citrate buffer, pH 4.5). The pathogenesis of diabetes in this rat model is most likely similar to the pathogenesis in humans characterized by both insulin resistance and compensatory hyperinsulinemia (Popov, Simioneseu, & Shepherd, 2003), and hence this model was used. Animals had free access to food and water after the STZ injection. Both STZ-injected and STZ-noninjected animals continued on their original diet for the duration of the study. Rats with blood glucose level \( \geq 126 \) mg/dl at 0 min or \( \geq 199.8 \) mg/dl at 120 min were considered to be diabetic and used for the study.

**Experimental Groups**

The animals were randomly divided into 5 groups of 6 animals each. Group I served as control receiving olive oil as vehicle. Group II rats were given HFD for 2 weeks and then induced with STZ (35 mg/kg body weight in 0.1 M citrate buffer, pH 4.5; Srinivasan et al., 2005), and served as diabetic control rats. Group III diabetic rats were treated with SA at a dosage of 200 mg/kg body weight for 4 weeks (Haseena Banu, Kaladevi, Shanthi, & Sachdanandam, 2011). Group IV diabetic rats were treated with the standard drug MET at a dosage of 500 mg/kg body weight for 4 weeks. Group V control rats were given SA alone at a dosage of 200 mg/kg body weight for 4 weeks and served as drug control group.

**Biochemical Analysis**

After 30 days of treatment, the animals were sacrificed under chloroform anesthesia. The blood was collected and serum was separated and used for biochemical estimations. Liver and skeletal muscle tissues were quickly excised off, a portion of liver and skeletal muscle tissues was washed with saline, and their homogenates were prepared by using 0.1 M phosphate buffer, pH 7.4. The homogenates were centrifuged and the supernatant was used for the study. Glucose was estimated by the method of Trinder (1969) using a reagent kit. Oral glucose tolerance test (OGTT) was performed by the method of Du Vigneaud and Karr (1925). Insulin was
assayed by the solid-phase enzyme-linked immunosorbent assay (ELISA). Glycosylated hemoglobin (HbA1C) was estimated by the method of Rao and Pattabiraman (1990). Homeostatic model assessment for insulin resistance (HOMA-IR) and homeostatic model assessment for β-cell function (HOMA-β) were performed by the method of Mathews et al. (1985). The analysis of nonenzymatic antioxidants such as vitamin C (ascorbic acid) was done according to the method of Omaye, Turnbull, and Sauberlich (1979), vitamin E by the method of Quaife and Dju (1948), and glutathione (GSH) by the method of Moron, Depierre, and Mannervik (1979). The enzymatic antioxidants such as superoxide dismutase (SOD) was estimated by the method of Marklund and Marklund (1974), catalase (CAT) by the method of Sinha (1972), glutathione peroxidase (GPx) by the method of Rotruck et al. (1973), and glutathione S-transferase (GST) by the method of Habig, Pabst, and Jacob (1973). The activities of pathophysiological enzymes such as aspartate transaminase (AST), alanine transaminase (ALT), and alkaline phosphatase (ALP) were assayed by the method of King (1965a).

Statistical Analysis

Results were represented as mean ± SEM of 6 rats. Statistical significance between the experimental groups was assessed by using the Student’s t-test and one-way analysis of variance (ANOVA).

RESULTS

Table 1 depicts the levels of blood glucose, plasma insulin, HOMA-IR, and HOMA-β in control and experimental groups of rats. Group II rats showed a significant (p < .05) increase in the levels of glucose and alterations in HOMA-IR and HOMA-β. Upon treatment with the drug SA, Group III rats showed a decrease in blood glucose levels when compared with Group II rats. No changes were observed in Group V rats. No significant alterations were observed in insulin level between Group II rats when compared with Group I rats. And also improvements in the aberrations in HOMA-IR and HOMA-β were observed in Group III rats.

Table 2 depicts the levels of Hb and HbA1c in control and experimental groups of rats. Group II rats exhibited a significant decrease (p < .05) in Hb levels and an

| TABLE 1. Effect of SA on Fasting Glucose, Insulin, HOMA-IR, and HOMA-β in Control and Experimental Animals |
|-----------------|-----|-----|-----|-----|-----|
| Parameters      | Group I      | Group II     | Group III    | Group IV    | Group V     |
| Glucose (mg/dl) | 113 ± 5.20   | 256 ± 16.32  | 127 ± 12.56  | 146 ± 19.63 | 117 ± 5.88  |
| Insulin (µU/ml) | 21.56 ± 0.99 | 16 ± 2.35   | 27 ± 2.13   | 17 ± 1.68   | 20.6 ± 1.9   |
| HOMA-IR         | 4.52         | 8.91  0.05   | 6.28  0.05   | 4.73  0.05  | 4.2  0.05   |
| HOMA-β          | 461.69       | 47.12  0.05  | 331  0.05    | 130.8  0.05 | 378.6  0.05 |

Note: Values are expressed as mean ± SD for 6 animals. Group I = control; Group II = diabetes induced; Group III = diabetes induced + SA treated (200 mg); Group IV = diabetes induced + MET treated (500 mg); Group V = control + SA alone (200 mg/kg body weight).

Control vs. other groups.

DC vs. DC + SA, DC + MET, and SA.

*p < .05; **NS, nonsignificant.
increase in HbA1c levels. Treatment with the drug SA for 30 days in Group III rats resulted in an increase in Hb and a decrease in HbA1c levels.

OGTT acts as a more sensitive measure to assess the early abnormalities in glucose regulation, and its alterations have been shown in Table 3. OGTT was impaired in Group II rats when compared with Group I rats. In our study, oral administration of both SA and MET to diabetic rats showed a significant reduction in peak blood glucose level at 1 and 2 h in Groups III and IV rats during OGTT.

The alterations in the activities of marker enzymes in the liver and serum of control and experimental groups of rats have been depicted in Table 4 and Figure 1, respectively. The activities of AST, ALT, ALP, ACP, and LDH were increased significantly ($p < .05$) in the serum and liver of diabetic group of rats when compared with control group of rats. Upon treatment with SA as well as MET, the activities of these enzymes were restored to near normalcy in Groups III and IV rats when compared with control (Group I) group of rats.

Tables 5 and 6 show the activities of SOD, CAT, GPx, and GST in the liver and skeletal muscle tissues of control and experimental groups of rats. During diabetes, there was a significant reduction ($p < .05$) in the activities of these enzymes in the tissues of Group II rats. Treatment with SA and MET restored the activities of these enzymes to near-normal levels in Groups III and IV rats.

The levels of nonenzymatic antioxidants such as vitamin C, vitamin E, and GSH, and also thiol and nonprotein thiol groups in the liver and skeletal muscle tissues of control and experimental groups of rats are shown in Tables 7 and 8. A significant ($p < .05$) decrease in the levels of vitamin C, vitamin E, and reduced GSH were observed in the liver and skeletal muscle tissues of HFD STZ-induced diabetic rats.

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**TABLE 2. Effect of SA on Hb and HbA1c in Control and Experimental Animals**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
<th>Group V</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb (g/dl)</td>
<td>12 ± 0.39</td>
<td>7.94 ± 0.28&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.74 ± 1.53&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>11.99 ± 1.23&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>10.65 ± 1.06&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>HbA1c (g/dl)</td>
<td>5.4 ± 0.26</td>
<td>13 ± 2.27&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.37 ± 0.88&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>7.2 ± 0.61&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>5.08 ± 0.26&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Note: Values are expressed as mean ± SD for 6 animals. Group I = control; Group II = diabetes induced; Group III = diabetes induced + SA treated (200 mg); Group IV = diabetes induced + MET treated (500 mg); Group V = control + SA alone (200 mg/kg body weight).

<sup>a</sup>Control vs. other groups.
<sup>b</sup>DC vs. DC + SA, DC + MET, and SA.
<sup>∗</sup>p < .05; <sup>**</sup>NS, nonsignificant.

---

**TABLE 3. Effect of SA on OGTT in Control and Experimental Animals**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
<th>Group V</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 min</td>
<td>82.5 ± 5.4</td>
<td>280.7 ± 14.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>148 ± 10.4&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>121.5 ± 8.2&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>83 ± 5.8&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>60 min</td>
<td>171.14 ± 11.6</td>
<td>352.6 ± 24.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>230.7 ± 17.8&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>197.8 ± 15.4&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>160.6 ± 11.9&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>120 min</td>
<td>129.7 ± 9.4</td>
<td>300.5 ± 21.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>170.6 ± 10.7&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>158.4 ± 7.8&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>130.1 ± 1.3&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Note: Values are expressed as mean ± SD for 6 animals. Group I = control; Group II = diabetes induced; Group III = diabetes induced + SA treated (200 mg); Group IV = diabetes induced + MET treated (500 mg); Group V = control + SA alone (200 mg/kg body weight).

<sup>a</sup>Control vs. other groups.
<sup>b</sup>DC vs. DC + SA, DC + MET, and SA.
<sup>∗</sup>p < .05; <sup>**</sup>NS, nonsignificant.
TABLE 4. Effect of SA on Activities of Marker Enzymes in the Liver of Control and Experimental Animals

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
<th>Group V</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST</td>
<td>11.05 ± 0.97</td>
<td>51.26 ± 2.51&lt;sup&gt;a&lt;/sup&gt;</td>
<td>29.84 ± 1.55&lt;sup&gt;b&lt;/sup&gt;</td>
<td>16.37 ± 1.21&lt;sup&gt;b&lt;/sup&gt;</td>
<td>11.10 ± 1.01&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>ALT</td>
<td>29.31 ± 1.36</td>
<td>90.32 ± 5.32&lt;sup&gt;a&lt;/sup&gt;</td>
<td>48.32 ± 2.54&lt;sup&gt;b&lt;/sup&gt;</td>
<td>35.08 ± 1.98&lt;sup&gt;b&lt;/sup&gt;</td>
<td>29.27 ± 2.12&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>ALP</td>
<td>6.18 ± 0.33</td>
<td>24.38 ± 1.33&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15.80 ± 1.21&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.34 ± 0.55&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.21 ± 0.45&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>ACP</td>
<td>4.75 ± 0.26</td>
<td>24.38 ± 1.33&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14.31 ± 0.95&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.66 ± 0.61&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.71 ± 0.19&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>LDH</td>
<td>0.86 ± 0.04</td>
<td>21.56 ± 1.87&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.14 ± 0.11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.01 ± 0.06&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.83 ± 0.03&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Note: Units: AST & ALT in µmol of pyruvate liberated/min/mg protein and ALP & ACP in µmol of phenol liberated/min/mg protein. Values are expressed as mean ± SD for 6 animals. Group I = control; Group II = diabetes induced; Group III = diabetes induced + SA treated (200 mg); Group IV = diabetes induced + MET treated (500 mg); Group V = control + SA alone (200 mg/kg body weight).
<sup>a</sup>Control vs. other groups.
<sup>b</sup>DC vs. DC + SA, DC + MET, and SA.
<sup>*p < .05; **NS, nonsignificant.

After administration of SA to diabetic rats (Group III), all the values were restored to near-normal levels when compared with Group I rats.

**DISCUSSION**

T2DM is a chronic metabolic disorder characterized by insulin resistance in the liver, muscle, and adipose tissue, as well as progressive β-cell dysfunction leading to hyperglycemia (Taniguchi, Emanuelli, & Kahn, 2006). The search for a safe and effective drug that can reduce the many harmful effects of T2DM, including hyperglycemia, hyperlipidemia, oxidative stress, inflammation, and arthrosclerosis, among others, is thus urgently needed. Hence, we have tried to bring about the antidiabetic and antioxidant potential of the drug SA in an HFD STZ-induced T2DM rat model. An HFD with STZ for induction of T2DM in the rat has been found to be a better model for diabetes research. The pathogenesis of diabetes in the rat model is most likely similar to the pathogenesis in humans characterized by both insulin resistance and compensatory hyperinsulinemia (Popov et al., 2003).

TABLE 5. Effect of SA on Activities of Enzymatic Antioxidants in the Liver of Control and Experimental Animals

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
<th>Group V</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOD</td>
<td>81.44 ± 5.36</td>
<td>52.37 ± 3.87&lt;sup&gt;a&lt;/sup&gt;</td>
<td>71.64 ± 4.32&lt;sup&gt;b&lt;/sup&gt;</td>
<td>79.32 ± 4.79&lt;sup&gt;b&lt;/sup&gt;</td>
<td>81.48 ± 5.45&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>GPx</td>
<td>4.12 ± 0.26</td>
<td>2.55 ± 0.18&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.47 ± 0.23&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.01 ± 0.31&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.15 ± 0.19&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>CAT</td>
<td>53.40 ± 3.84</td>
<td>29.42 ± 1.57&lt;sup&gt;a&lt;/sup&gt;</td>
<td>41.26 ± 2.12&lt;sup&gt;b&lt;/sup&gt;</td>
<td>50.85 ± 3.61&lt;sup&gt;b&lt;/sup&gt;</td>
<td>53.45 ± 2.68&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>GST</td>
<td>3.28 ± 0.21</td>
<td>2.09 ± 0.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.72 ± 0.22&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.14 ± 0.15&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.31 ± 0.25&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>GR</td>
<td>6.05 ± 0.43</td>
<td>4.37 ± 0.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.54 ± 0.38&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.91 ± 0.41&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.08 ± 0.54&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>LPO</td>
<td>1.04 ± 0.08</td>
<td>2.14 ± 0.19&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.93 ± 0.21&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.54 ± 0.42&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.03 ± 0.09&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Note: Units: SOD in U/min/mg protein; CAT in µmol of H<sub>2</sub>O<sub>2</sub> consumed/min/mg protein; GPx in µmol of GSH oxidized/min/mg protein; GST in µmol of CDNB utilized/min/mg protein; GR in nmol of GSSG reduced/min/mg protein. Values are expressed as mean ± SD for 6 animals. Group I = control; Group II = diabetes induced; Group III = diabetes induced + SA treated (200 mg); Group IV = diabetes induced + MET treated (500 mg); Group V = control + SA alone (200 mg/kg body weight).
<sup>a</sup>Control vs. other groups.
<sup>b</sup>DC vs. DC + SA, DC + MET, and SA.
<sup>*p < .05; **NS, nonsignificant.
Diabetes is usually associated with hyperglycemia, impaired glucose tolerance (IGT), and also increased glycation of hemoglobin. Hyperglycemia is a characteristic feature of diabetes mellitus resulting from increased hepatic glucose production and decreased utilization by peripheral tissues, and this was reflected in our study also. The normal blood glucose level in rats is 50–135 mg/dL (Linda, 1995). Rats fed with HFD and induced with STZ (Group II rats) had no variation in insulin levels when compared with control Group I rats. The observed decrease in blood glucose levels might be due to the hypoglycemic effect of the drug, while at the same time, the increase in insulin levels might be due to the protective effect of the flavonoids present in the drug on the β cells of the pancreas (Arul et al., 2004).

Hyperglycemia results in increased glycosylation of a number of proteins, including hemoglobin and β crystalline of the eye lens, and hence, the estimation of HbA1c has been found to be particularly useful in monitoring the effectiveness of therapy in diabetes (Goldstein, 1995). The observed reduction in the levels of HbA1c might be due to the free radical quenching property of the drug since agents with antioxidant or free radical scavenging properties are capable of inhibiting

### TABLE 6. Effect of SA on Activities of Enzymatic Antioxidants in the Skeletal Muscle of Control and Experimental Animals

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
<th>Group V</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOD</td>
<td>55.28 ± 3.57</td>
<td>31.40 ± 3.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>45.76 ± 3.81&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>52.63 ± 4.05&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>55.25 ± 4.85&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>GPx</td>
<td>3.56 ± 0.21</td>
<td>2.12 ± 0.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.92 ± 0.18&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>3.40 ± 0.29&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>3.52 ± 0.15&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>CAT</td>
<td>34.41 ± 2.77</td>
<td>18.94 ± 1.76&lt;sup&gt;a&lt;/sup&gt;</td>
<td>28.08 ± 2.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>32.96 ± 2.84&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>34.43 ± 3.02&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>GST</td>
<td>2.25 ± 0.12</td>
<td>1.51 ± 0.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.92 ± 0.16&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.13 ± 0.09&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>2.28 ± 0.25&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>GR</td>
<td>4.32 ± 0.31</td>
<td>3.25 ± 0.27&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.82 ± 0.34&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>4.20 ± 0.38&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>4.34 ± 0.29&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>LPO</td>
<td>1.02 ± 0.12</td>
<td>1.65 ± 0.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.27 ± 0.10&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>1.11 ± 0.14&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>1.09 ± 0.09&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*Note: Units: SOD in U/min/mg protein; CAT in μmol of H₂O₂ consumed/min/mg protein; GPx in μmol of GSH oxidized/min/mg protein; GST in μmol of CDNB utilized/min/mg protein; GR in nmol of GSSG reduced/min/mg protein. Values are expressed as mean ± SD for 6 animals. Group I = control; Group II = diabetes induced; Group III = diabetes induced + SA treated (200 mg); Group IV = diabetes induced + MET treated (500 mg); Group V = control + SA alone (200 mg/kg body weight).<sup>a</sup>Control vs. other groups.<sup>b</sup>DC vs. DC + SA, DC + MET, and SA.<sup>p</sup><sup>∗</sup>p < .05; <sup>∗∗</sup>NS, nonsignificant.

### TABLE 7. Effect of SA on Activities of Nonenzymatic Antioxidants in the Liver of Control and Experimental Animals

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
<th>Group V</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSH</td>
<td>3.55 ± 0.22</td>
<td>2.37 ± 0.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.98 ± 0.24&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>3.32 ± 0.29&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>3.49 ± 0.15&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>Total thiol (T-SH)</td>
<td>6.09 ± 0.54</td>
<td>4.12 ± 0.31&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.23 ± 2.04&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>5.99 ± 0.43&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>6.11 ± 0.52&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>Nonprotein thiol (NP-SH)</td>
<td>2.68 ± 0.19</td>
<td>1.78 ± 0.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.26 ± 0.21&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>2.54 ± 0.17&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>2.70 ± 0.23&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>1.21 ± 0.08</td>
<td>0.67 ± 0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.98 ± 0.06&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>1.15 ± 0.09&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>1.25 ± 0.05&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>3.98 ± 0.25</td>
<td>2.07 ± 0.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.39 ± 0.27&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>3.81 ± 0.18&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>4.01 ± 0.34&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*Note: Units: μg/mg protein. Values are expressed as mean ± SD for 6 animals. Group I = control; Group II = diabetes induced; Group III = diabetes induced + SA treated (200 mg); Group IV = diabetes induced + MET treated (500 mg); Group V = control + SA alone (200 mg/kg body weight).<sup>a</sup>Control vs. other groups.<sup>b</sup>DC vs. DC + SA, DC + MET, and SA.<sup>p</sup><sup>∗</sup>p < .05; <sup>∗∗</sup>NS, nonsignificant.
oxidative reactions associated with protein glycation (Elgawish, Glomb, Freeland, & Monnier, 1996).

Group II rats showed an IGT when compared with Group I of rats. This might have resulted from increased hepatic gluconeogenesis and reduced uptake of glucose from the blood into the skeletal muscle and adipose tissue following a meal, and hence, it serves as a marker for the state of insulin resistance and predicts both large- and small-vessel vascular complications (Tominaga et al., 1999). SA might have enhanced glucose utilization by peripheral tissues and also by increasing the glycogen stores in the liver and skeletal muscle.

Studies have shown that both high HOMA-IR and low HOMA-β are associated with increased prevalence of IGT and Type 2 diabetes (Osei, Rhinesmith, Gaillard, & Schuster, 2004). The observed alterations in these parameters established the potential antidiabetic effect of the drug.

The elevation in the activities of enzymes ALT, AST, ALP, and ACP are hypothesized as predictors of diabetes (Elizabeth & Harris, 2005). Both HFD and STZ are known to cause injury to the liver. High-fat feeding is usually associated with fatty liver, thereby resulting in the long-term elevations of liver enzymes (Clark, Brancati, & Diehl, 2003). Liver is bombarded by the free fatty acids (FFA) that pour out of the adipose tissue into the portal blood, which can directly cause inflammation within the liver cells and then release further proinflammatory cytokines, leading to more hepatocyte injury and affecting the integrity of liver cells (Fielding & Frayn, 2000). This also indicates impairment in insulin signaling, since insulin is involved in the suppression of transcription of these gluconeogenic enzymes rather than purely hepatocyte injury (O’Brien & Granner, 1991). The insulin-resistant state characterized by an increase in proinflammatory cytokines such as tumor necrosis factor-α (TNF-α) may also contribute to hepatocellular injury (Palsamy & Subramanian, 2008). Elevation in the activities of ALT, AST, and ALP in the diabetic rats may be due to the leakage of these enzymes from the liver cytosol into the bloodstream as a consequence of the hepatotoxic effect of STZ, which is consistent with other studies (Chuanxia et al., 2010). The decrease in the levels of marker enzymes in Group III rats suggests the tissue protective nature of SA. This may be due to the presence of flavonoids in the drug. Flavonoids are known to have anti-inflammatory properties by inhibiting the series of enzymes that are activated during the inflammatory

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
<th>Group V</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSH</td>
<td>2.06 ± 0.12</td>
<td>1.12 ± 0.07²</td>
<td>1.68 ± 0.09²</td>
<td>1.92 ± 1.19²</td>
<td>2.04 ± 0.21²</td>
</tr>
<tr>
<td>Total thiol (T-SH)</td>
<td>3.94 ± 0.23</td>
<td>2.03 ± 0.16²</td>
<td>3.19 ± 0.24²</td>
<td>3.81 ± 0.21²</td>
<td>3.97 ± 0.26²</td>
</tr>
<tr>
<td>Nonprotein thiol (NP-SH)</td>
<td>1.91 ± 0.06</td>
<td>1.04 ± 0.09²</td>
<td>1.49 ± 0.07²</td>
<td>1.82 ± 0.08²</td>
<td>1.95 ± 0.16²</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>0.95 ± 0.04</td>
<td>0.50 ± 0.03²</td>
<td>0.71 ± 0.05²</td>
<td>0.86 ± 0.07²</td>
<td>0.98 ± 0.08²</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>2.64 ± 0.17</td>
<td>1.71 ± 0.09²</td>
<td>2.28 ± 0.06²</td>
<td>2.55 ± 0.21²</td>
<td>2.66 ± 0.24²</td>
</tr>
</tbody>
</table>

*Note: Units: µg/mg protein. Values are expressed as mean ± SD for 6 animals. Group I = control; Group II = diabetes induced; Group III = diabetes induced + SA treated (200 mg); Group IV = diabetes induced + MET treated (500 mg); Group V = control + SA alone (200 mg/kg body weight).

²Control vs. other groups.

*p < .05; **NS, nonsignificant.
process (González-Gallego, Sánchez-Camposy, & Tuñón, 2007), thereby preventing hepatocellular damage.

Reactive oxygen species (ROS) are chemically reactive molecules containing oxygen. Examples include oxygen ions and peroxides. ROS are highly reactive due to the presence of unpaired valence shell electrons (Devasagayam et al., 2004). Oxidative stress, through the production of ROS, has been proposed as the root cause underlying the development of insulin resistance and β-cell dysfunction, thereby inducing T2DM (Wright, Scism-Bacon, & Glass, 2006). In hyperglycemic condition, the autoxidation of glucose increases the formation of free radicals beyond the capacity of defense system to neutralize it and thus causes oxidative stress (Sharma, Mishra, Ajmera, & Mathur, 2005). The removal of free radicals is important because free radicals cause oxidative damage to membrane lipids (LPO), proteins (protein carbonyl [PC] formation), and nucleic acid molecules (Gutteridge, 1995). Several studies have shown increased levels of LPO in tissues of Type 2 diabetic rats (Li et al., 2010). There is extensive evidence suggesting that LPO and protein oxidation lead to loss of membrane integrity, an important factor in acceleration of diabetes mellitus (Maritim, Dene, Sanders, & Watkins, 2003). LPO is frequently used as an index of tissue oxidative stress. It is a free-radical-mediated reaction of oxidative insult to polyunsaturated fatty acids involving several types of free radicals, and termination occurs through enzymatic means or through free radical scavenging by antioxidants (Korkina & Afanas’ev, 1997). Elevated levels of free radicals generated from carbohydrate autoxidation and reactive carbonyl compounds lead to protein damage during diabetes. Administration of SA decreased the LPO levels by enhancing the antioxidant defense system, which may be due to the antioxidant property of the drug (Ramprasath, Shanthi, & Sachdanandam, 2005).

The thiol group present in the amino acid cysteine can be found in many cellular peptides or proteins and can be oxidized to form disulfide. These thiol antioxidative peptides or proteins play an important role in the cellular antioxidative defense as well as in the regulation of cellular functions involving the thiol–disulfide exchange. Protein-bound thiols were found to be lower in Type 2 diabetic patients compared with their controls (Ceriello et al., 1997). The diminished protein-SH group observed in diabetic rats signifies protein damage resulting from oxidative stress. Drugs with antioxidant properties may increase endogenous defense systems and reduce both initiation and propagation of ROS (Bergendi, Benes, Durackova, & Ferencik, 1999). SA effectively reduced the increased levels of the total and protein thiols and PC content in diabetic rats, which affirms the notion that SA has free radical scavenging potential in the diabetic state. This effect may be attributed to the free radical quenching activity of flavonoids (Dias et al., 2005). The 4-oxo group present in all the biflavanones in SA contributes to the free radical quenching activity. The 5-OH and 7-OH groups present in jeediflavanone also confer scavenging potential. Semecarpufuflavanone, semecarpetin, and galluflavanone have a 7-OH group that might contribute to the free radical chelating activity of SA (Umarani, Shanthi, & Sachdanandam, 2008).

Diabetic (Group II) animals showed a significant decrease in the activities of antioxidant enzymes when compared with control animals, while SA-treated (Group III) animals showed a significant increase in the activities of antioxidant enzymes when compared with diabetic (Group II) animals. Antioxidants are the major
defense systems that limit the toxicity associated with free radicals. The levels of these are altered in diabetes, and hence, the ineffective scavenging of free radicals plays a crucial role in determining the extent of tissue injury (Ramachandran, Ravi, Narayanan, Kandaswamy, & Subramanian, 2004). Increased oxidative stress is more common in organs and tissues with high metabolic and energy demands, including skeletal and liver. The activities of SOD, GSH, and GPx in diabetic subjects and in skeletal muscle tissue may be reduced (Matkovics, Varga, Szabo, & Witas, 1982), whereas in other tissues, including kidneys and thymus, antioxidant enzymes’ activities are in fact increased. In both human and experimental animal models of Type 2 diabetes, high oxidative stress exists due to persistent and chronic hyperglycemia, which depletes the activity of free radical scavenging enzymes and thus promotes the generation of free radicals (Palanisamy, Govindasamy, Ganesan, & Raman, 2009).

SOD and CAT are considered as primary antioxidant enzymes since they are involved in the direct elimination of ROS. A decrease in the activity of these antioxidants can lead to an excess availability of superoxide anion (O$_2^{-}$) and hydrogen peroxide (H$_2$O$_2$), which in turn generate hydroxyl radicals (•OH), resulting in the initiation and propagation of LPO. SOD is an important defense enzyme, which catalyzes the dismutation of superoxide radicals and thereby reducing the likelihood of superoxide anion interacting with NO to form reactive peroxynitrite (Maritim et al., 2003). The hydrogen peroxide is then successively metabolized into water and non-ROS by the activities of CAT and GPx (Matés & Sánchez-Jiménez, 1999). CAT, a tetrameric hemin enzyme, catalyzes the reduction of hydrogen peroxides and protects tissues from highly reactive hydroxyl radicals (Chance, Greenstein, & Roughton, 1952). The elevated production of hydrogen peroxide due to the autoxidation of glucose, protein glycation, and LPO might have lead to the marked decline in the CAT activity in diabetic rats (Matés & Sánchez-Jiménez, 1999).

GPx is a selenium-containing enzyme, which catalyses the detoxification of hydrogen peroxide and lipid peroxide by using GSH as a hydrogen donor and acts as a peroxynitrite reductase (Inefers & Sies, 1988). Decreased activity of GPx, observed in diabetic liver and skeletal muscle tissue, has been shown to be an important adaptive response to increased peroxidative stress (Matkovics et al., 1982).

GST catalyzes the conjugation of GSH to a wide range of electrophiles and supports a protective mechanism against hyperglycemia-mediated oxidative stress. It is critical in the protection of cells from reactive species because they utilize a wide variety of products of oxidative stress as substrates (Bekris et al., 2005). The decreased level of glutathione synthetase, observed in diabetic animals, represents an increased utilization resulting from oxidative stress (Anuradha & Selvam, 1993).

Along with the increased generation of free radicals, ruined antioxidant competence of nonenzymatic antioxidants such as vitamin E, vitamin C, and GSH has been reported in diabetes (Davì, Falco, & Patrono, 2005). The nonenzymatic antioxidant enzymes detoxify free radicals directly and also interact in recycling processes to engender the reduced forms of the nonenzymatic antioxidants. Vitamin E is a fat-soluble vitamin that is sequestered in the hydrophobic interior of membranes where it acts as an antioxidant to quench LPO by directly reacting with peroxyl and superoxide radicals and singlet oxygen (Jessup, Dean, De Whalley, Rankin, & Leake, 1990). Vitamin C is an excellent hydrophilic antioxidant in plasma and
disappears faster than other antioxidants on exposure to ROS (Inefers & Sies, 1988). A decrease in the levels of vitamin C in diabetic rats may be due to either an increased utilization as an antioxidant defense against increased ROS or a decrease in GSH level, since GSH is required for the recycling of vitamin C (Jin, Shao, Wang, Chen, & Jin, 2000). GSH is a chief intracellular redox component that functions as a direct free radical scavenger and is a co-substrate for the GPx activity and also for many enzymes. GSH facilitates the rearrangement of dicarbonyl to hydroxy acids and entraps reactive unsaturated aldehydes into nonreactive hydrophilic Michael adducts that are easily excretable by the cell (Aldini, Dalle-Donne, Facino, Milzani, & Carini, 2007). Vitamin C in combination with vitamin E counters free radicals by inhibiting hydroperoxide formation and Fenton-type reactions, and also regulates vitamin E metabolism by recycling oxidized tocopherols. The synergistic action of these enzymes is also modulated by the intervention of GSH, which maintains vitamin C in the reduced form (Chow, 1991).

These effects indicate that SA may scavenge or inhibit free radical formation and participate in stabilizing the endogenous antioxidant network, including GSH, and concomitantly decrease LPO in various conditions caused by free radicals. This may be attributed to the presence of phenolic compounds such as flavonoids in the drug. The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides. Flavonoids have been shown to decrease the LPO and increase the antioxidant enzyme activity in Type 2 diabetic patients.

CONCLUSION

In conclusion, our results showed that through its antioxidant properties, SA enhanced endogenous GSH levels and thereby conferred protection against HFD STZ-induced T2DM in rats. The study also suggests that SA is effective in preventing diabetic complications such as hyperglycemia and oxidative damage. Thus, SA may be considered as a potential candidate in the treatment of diabetes mellitus. Further investigations are being carried out to trace the exact mechanistic pathways.

Declaration of interest: The authors report no conflict of interest. The authors alone are responsible for the content and writing of this article.

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