Salvia fruticosa-mediated antioxidant protection against oxidative stress in streptozotocin – induced diabetic rats: Role of α-glucosidase activity

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ABSTRACT

Oxidative stress is involved in the development and progression of diabetic complications. Salvia fruticosa was reported to have a protective action against oxidative stress. In the present study, diabetes was induced in male Wister rats using streptozotocin (60 mg/kg body weight). Diabetic rats exhibit not only the symptoms of diabetes like the loss of body weight, hyperglycemia, polyuria and polydipsia but oxidative stress. Daily ingestion of 1gm/kg of body weight of Salvia fruticosa extract for 60 days to diabetic rats reversed the adverse effect of diabetes on rats. Salvia fruticosa extract provides better control of glucose levels and body weight loss. In heart, kidney, and liver, diabetes-induced changes in lipid peroxidation marker (malondialdehyde) were entirely attenuated by Salvia fruticosa treatment. This was accompanied by a significant decrease in reduced glutathione and superoxide dismutase activity and increase in catalase activity in these tissues. Results from this study indicate that Salvia fruticosa extract modulates the adverse effect of diabetes on streptozotocin-induced diabetic rats. SF extract was evaluated for in vitro antioxidant capacity by estimating of total phenolics content, the ferric reducing antioxidant power (FRAP) assay, 1,1-diphenyl-2-picrylhydrazyl (DPPH), and ABTS assays. The extract was found to inhibit α-glucosidase activity similar to acarbose. Salvia fruticosa extract may have a potential therapeutic effect due to its antioxidant and anti-hyperglycemic properties.

KEY WORDS

Salvia fruticosa, Antioxidants capacity, α-glucosidase activity, Oxidative stress, Diabetic rats.

INTRODUCTION

Diabetes-induced oxidative stress is the key factor responsible for the onset of its complications. There is growing evidence that excess generation of reactive oxygen species (ROS), largely due to hyperglycemia, causes oxidative stress in a variety of tissues of diabetic patients. One of the main goals of treatment of diabetes is to prevent its complications and several studies confirmed that antioxidant treatment reduces diabetic complications. The antioxidant protective effect of natural plants is promising therapeutic drugs for free radical pathologies. Among natural plants, Salvia fruticosa Mill (SF) and other Salvia species (Lamiaceae) are found to have antioxidant effect. SF (Salvia triloba) is a native plant of the Mediterranean, which has been used in traditional medicine.
medicine by many Asian and Middle Eastern countries to treat several common healthy complications such as cold, headaches, and abdominal pain\textsuperscript{15-16}. The aqueous and oil extracts of SF have been shown to possess antioxidant, anti-inflammatory, anticancer and antimicrobial activities\textsuperscript{16}. Salvia species are generally known for their multiple pharmacological effects including their anti-bacterial\textsuperscript{17}, anti-hyperglycaemic\textsuperscript{18-20}, anti-cancer\textsuperscript{21} and anti-cholestatic\textsuperscript{22} effects. However, the extract of SF has not received much attention in comparison to Salvia officinalis extracts. Phytochemically, salvia species contain several phenolic compounds that prevent peroxidative damage to tissues such as salvianolic acids, rosmarinic acid, carnosol, caffeic acid tanshinone IIA, and another phenolic glycosides\textsuperscript{23-26}. Considering the importance of oxidative stress in the pathophysiology of diabetic complications, the purpose of this study was to characterize changes in antioxidative defence and oxidative stress in the heart, liver and kidney of diabetic rats and to evaluate whether these changes can be prevented or attenuated by ethanolic extract of SF. Experimental diabetes was induced by administering a single streptozotocine (STZ) dose. SF protection was assessed via monitoring the marker of lipid peroxidation (LP) in tissues (malondialdehyde (MDA)), endogenous antioxidants (reduced glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) enzyme activities) and total antioxidant capacity (TAC). The in vitro antioxidant properties of SF were achieved through investigating the radical-scavenging effects, including DPPH, and ABTS assays and the ferric reducing antioxidant power (FRAP) assay.

**MATERIALS AND METHODS**

**Chemicals**

Thiobarbituric acid, reduced glutathione, 5,5-dithiobis (2-nitrobenzoic acid), Folin’s reagent, epinephrine, SOD enzyme, H2O2, Streptozotocin and bovine albumin 2,4,6-tripyridyltriazine, gallic acid, sodium carbonate, 1,1-diphenyl-2-picrylhydrazyl (DPPH), the α-glucosidase enzyme from Saccharomyces cerevisiae, 4-nitrophenol α-D-glucopyranoside were obtained from Sigma Chemical Co. (St. Louis, MO). All other chemicals purchased from common commercial suppliers.

**Preparation of Plant Extract:**

Fresh SF was collected during the flowering period in March and April 2015 from southern Mediterranean region at altitudes ranging from 200 to 400 m at the littoral of Beirut, Lebanon. The plant was dried under shade at 25°C and the dried aerial parts were purchased from a local herbal store (Cairo, Egypt). The plant was botanically authenticated by Dr. Nael M. Fawzi, Flora and Taxonomy Department, Agricultural Research Center, Giza, Egypt. A voucher specimen was deposited at the herbarium of the Flora and Taxonomy Department, Giza, Egypt.

To increase the yield of extraction in a shorter time and a lower temperature, the liquid-phase microwave-assisted process was used for extraction of SF according to the methods described by Pan et al.\textsuperscript{27} and Alfaro et al.\textsuperscript{28}. These microwave-assisted extraction applications are based upon the selective and localized heating of particular components within the material being treated. When the material is immersed in a solvent that is transparent to microwaves (ethanol and water), the resulting increases in localized temperature and pressure cause the target compounds to migrate from the material to the relative cold solvent surrounding. The air-dried plant material was crushed in a grinder. A sample of 100 gms of ground-dried plants were extracted with 1000 ml of 70 % (v/v) aqueous ethanol. The extract was irradiated with microwave for two minutes. The extract was then filtered through gauze, after overnight maceration, and ethanol evaporated under reduced pressure at 40°C by using a rotary evaporator. Then the water extract was dried by using a freeze dry system under reduced pressure. The dried extract was dissolved in distilled water before administration to normal and diabetic animals.

**In vitro antioxidant properties**

Total antioxidant properties of SF were estimated by the total phenolic content (TPC) the ferric reducing antioxidant power (FRAP), 1,1-diphenyl-2-picrylhydrazyl (DPPH\textsuperscript{+}), 2, 2-azino-bis (3-ethylbenzothiazoline-6-sulfonate) (ABTS\textsuperscript{+}). TPC was determined by the method of 29 using the Folin-Ciocalteu reagent and expressed in milligrams of gallic acid equivalent per g dry weight of plant material. The FRAP assay was determined according to the method of Benzie 30 and DPPH and ABTS methods were determined as previously described 31. IC50 value (mg/mL) is the concentration of extract that causes
DPPH· radical is scavenged by 50%. In ABTS assay, the calibration curve of ascorbic acid was established, the antioxidant capacity of the SF was then expressed as μmol ascorbic acid equivalent/g dry extract.

**In vitro α-glucosidase inhibition assay**

The α-glucosidase enzyme inhibitory assay was performed in a 96 well plate as described earlier the papers 32. α-Glucosidase (25μl, 0.5 U/ml) in 100 mM potassium phosphate buffer (pH 6.8) were mixed with 25μl of test sample. Acarbose (1 mg/ml) was also assayed as a standard reference and incubated at 25 °C for 15 min. After the incubation 25 μL of the substrate4-nitrophenylα-D-glucopyranoside (1 mM concentration in 100 mM potassium phosphate buffer, pH 6.8) were added to the mixture. The enzymatic reaction was allowed to proceed at 25 °C for 10 min and was stopped by the addition of 100 μL of 0.2 M sodium carbonate. Nitrophenol absorption was measured at 405 nm using an Elisa Reader. The percent inhibition of α-glucosidase was calculated as (1-(Abs Sample-Abs blank)/Abs control) ×100, where Abs sample represents the absorbance of the experimental sample, Abs blank represents the absorbance of the blank, and Abs control represents the absorbance of the control. The IC50 value was defined as the concentration of α-glucosidase inhibitor that inhibited 50% of α-glucosidase activity. Nonlinear regression (with sigmoidal dose response) was used to calculate theIC50 values of using Origin Pro v8.

**Animals**

The Healthy adult male Wistar rats (8 weeks old, 130-180 g) were obtained from the animal house of the National Research Center (Giza, Egypt). They were maintained on standard pellet diet and tap water ad libitum and were kept in polycarbonate cages with wood chip bedding under a 12 hr light/dark cycle and room temperature 22-24°C. Rats were acclimatized to the environment for two weeks prior to experimental use. All experiments were carried out in accordance with research protocols established by the animal care committee of the National Research Center, Egypt, which followed the recommendations of the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985).

**Acute toxicity test**

The acute toxicity of the crude ethanolic extract of SF was determined by using male rats (150–200g), according to the method described by Ahmad et al., 33. Rats were randomly grouped into four groups and in per group; rats were doused with 250, 500 1000 and 2000 mg/kg of the SF extract orally via gastric gavage. All animals were observed for any kind of behavioral changes, physical and pharmacological toxic effects at 0, 30 and 60 min, 24, 48 and 72h and 1 week after administration. They were no observed over a period of one week for signs of toxicity and mortality.

**Induction of experimental diabetes**

Diabetes induced by injection a single intraperitoneal dose of STZ 60 mg/kg body weight and was freshly prepared in 0.01 M citrate buffer, pH 4.5 as described previously 34. The control rats were injected with the same volume of citrate buffer. STZ injected animals were given 20% glucose solution overnight to overcome drug induced hypoglycemic mortality. Diabetes was identified by polydipsia, polyuria and measuring blood glucose concentration 72 hours after injection of STZ. Blood samples were taken from the tail vein. The blood glucose level was determined by glucose oxidase method using a one touch basic plus glucometer (Lifescane Ltd., California, USA). Rats with a blood glucose level above 250 mg / dl were considered diabetic and were used in this study.

**Treatment schedule**

The rats were divided into three groups with 10 rats in each group and were treated through a gavage tube for a period of for 8 weeks as follows: The diabetic rats were randomly divided into two sub - groups with or without SF. SF was given orally by gavage at a dose level of one gm per kg of body weight, daily for 8 weeks. The control animals received equivalent volume of distilled water based on body weight.

**Sample Preparation**

After 8 weeks of SF or vehicle solution administration, animals were fasted overnight and blood glucose levels were determined using glucometer, and then the rats anaesthetized with diethyl ether anesthesia. The animals were sacrificed by cervical dislocation and one gm of heart, kidney and liver were removed and homogenized in ice-cold potassium chloride (150 mM). The ratio of tissue weight to homogenization buffer was 1:10. From the latter, suitable dilutions for determination of the levels of GSH, LP product MDA, total proteins, and activities of SOD and CAT were prepared in suitable different buffers.

**Biochemical Assays**
The GSH content in the tissue homogenate was determined using the method described by Van Dooran et al. 35. The basis of the GSH determination method is the reaction of Ellman’s reagent 5,5-dithiobis(2-nitrobenzoic acid) (DTNB) with thiol group of GSH at pH 8.0 to give yellow color of 5-thiol-2-nitrobenzoate anion.

Malondialdehyde is the most abundant individual aldehyde resulting from LP breakdown in biological systems and used as an indirect index of LP, the determination of MDA in biological materials, as described in Aksoy et al. 8, is based on its reaction with thiobarbituric acid (TBA) to form a pink complex with absorption maximum at 535 nm.

The total antioxidant capacity in tissues was evaluated using ferric reducing antioxidant power (FRAP) assay. The FRAP assay was determined according to the method described by Benzie and Strain. 30. The FRAP assay measures the change in absorbance at 593 nm due to the formation of a blue colored ferrous-tripyridyltriazine complex from colourless oxidized ferric form by the action of electron donating antioxidants.

The activity of SOD enzyme in tissue homogenates was determined according to the method described by Sun & Zigman. 36. This method is based on the ability of SOD to inhibit the auto-oxidation of epinephrine at alkaline pH to adrenochrome and other derivatives, which are easily monitored in the near-UV region of the absorption spectrum. CAT activity was determined by measuring the exponential disappearance of H2O2 at 240 nm and expressed as units/mg of protein as described by Aebi. 37.

The total protein content in tissues was determined according to the Lowry’s method modified by 38. In all the estimations, Absorbance was recorded using a PerkinElmer, Lambda 25 UV/VIS spectrophotometer in all measurements.

**Standardization of the aqueous extract using RP-HPLC**

Rosmarinic acid and carnosol were selected as a marker for HPLC standardization as it was the major bioactive compound we previously isolated from the SF. The dry residue of SF ethanol extract (20 mg) was dissolved in 10 mL of methanol and 20µl aliquot were analysis by HPLC. The separation was carried out on HPLC apparatus (Waters 600E system controller coupled with a photodiode array detector, Water 990 series). 20µl of aliquot were injected into a reverse phase NOVA-PAK C18, 20 x 3.9 mm i.d., particle size 420µm at 20 °C. The mobile phase was acetonitril (A) and acidified water containing 2.5% formic acid (B). The gradient was as follows: 0 min, 5% A; 10min, 15% A; 30 min, 25% A, 35 min, 30% A; 50 min, 55% A; 55 min 90% A; 60 min, 100% A and then hold for 10 min before returning to the initial conditions. The flow rate was 1.0 ml/ min and wavelengths were 280nm. Serial dilutions of standard of rosmarinic acid and carnosol were prepared from a stock solution having a final concentration. A standard calibration curve was established using the different concentrations. Each sample was injected in triplicates.

**Statistical Analysis**

The data are expressed as group mean ± SEM. SPSS (version 20) statistical program (SPSS Inc., Chicago, IL, USA) was used to carry out a one-way analysis of variance (ANOVA) on our data. When significant differences by ANOVA were detected, analysis of differences between the means of the treated and control groups were performed by using Dunnett’s t test.

**RESULTS**

**Acute toxicity studies**

Animals showed good tolerance to testing (250, 500, 1000 and 2000 mg/kg) doses of SF extract. Extract in doses as high as 2 g/kg that were found to be non-lethal. Highest dose of extract did not show any noticeable signs of toxicity and mortality after once daily administration orally for 7 days. Therefore, the extract is safe for long-term administration.

**Effect of SF on body weight change and blood glucose concentration in STZ-induced diabetic rats**

Diabetic rats exhibited decreased in final body weight and increased in blood glucose concentration when compared to the normal control group. Treatment with ethanol extract of SE effectively attenuated the decrease in body weight and the increase in blood glucose level in diabetic rats (Table 1).

**Table 1. Effect of SF on body weight changes and blood glucose in STZ-induced diabetic rats.**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Control</th>
<th>Diabetic</th>
<th>Diabetic + SF</th>
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</table>


Body weight changes (g) 60.00 ± 1.58 -19.00 ± 8.35*** 11.80 ± 5.52***
Blood glucose (mg/dl) 74.60 ± 0.60 435.60 ± 7.44*** 193.00 ± 37.24**

Dennett’s t test was performed after ANOVA test. Data are means ± SEM, ** P < 0.01, *** P < 0.001 vs. control.

Effect of SF on MDA and TAC in STZ-induced diabetic rats
STZ treated rats showed a significant increase in marker of LP. We measured LP as MDA. Diabetic control rats showed significantly elevated levels of MDA in heart (P < 0.001), liver (P < 0.01) and kidney (P < 0.001) tissues. SF completely prevented the elevation in MDA level (Figure 1 A). The TAC decreased in heart (P < 0.001), liver (P < 0.001) and kidney (P < 0.05) of diabetic rats. Whereas, the SF treatment prevented the depletion in TAC in diabetic animals (Figure 1 B).

Figure 1. Effect of treatment with SF on (A) MDA and (B) TAC in heart, liver and kidney of diabetic rats. Dennett’s t test was performed after ANOVA test.
Each column represents the mean ± SEM (n=6). * P<0.05, ** P<0.01, *** P<0.001 vs. control.
Effect of SF on CAT, SOD and GSH in STZ-induced diabetic rats

Although CAT activity was increased in heart (P < 0.05) and kidney (P < 0.05) of diabetic rats, there was a significant decrease (P < 0.01) in liver. The repeated treatment with SF completely prevented these changes in CAT (Figure 2 A). In STZ-treated rats, SOD activity was significant increase in heart (P < 0.01), liver (P < 0.001) and kidney (P < 0.01). While the repeated treatment with SF completely prevented this effect in diabetic rats (Figure 2 B). STZ induced elevation in cardiac (P < 0.05) and renal (P < 0.001) GSH levels while hepatic GSH content significantly decreased (P < 0.05). These changes in GSH contents were completely prevented by SF (Figure 2 C).

Figure 2. Effect of treatment with SF on (A) CAT, (B) SOD and (C) GSH in heart, liver and kidney of diabetic rats. Dennett’s t test was performed after ANOVA test. Each column represents the mean ± SEM (n=6). * P<0.05, ** P<0.01, *** P<0.001 vs. control.

In vitro antioxidant Activity of SF

In the present work, the TFC, FRAP, ABTS and DPPH• were used to test the antioxidant activities of SF extracts. The results of the three assays are summarized in Table 2. The TFC of SF was measured using Folin–Ciocalteau’s assay expressed in mg of gallic acid the plant extract has 25.5 ± 0.73 mg gallic acid/g. FRAP assay depends on reduction of oxidized ferric ions to ferrous ions by antioxidant. The reducing capacity of compound may serve as a significant indicator of its potential antioxidant activity. In the present study, each gram of dried SF has high FRAP value, 17.16 μmol ascorbic acid equivalent/g. Additionally, the ABTS and DPPH radical-scavenging methods are widely used for evaluating the ability of plant extracts to scavenge free radical generated from ABTS and DPPH reagents. The SF exhibited high anti free radical scavenging activity where the ascorbic acid equivalent antioxidant capacities of the SF were 30.16 ± 0.12 μmol /g in ABTS and The DPPH radical scavenging ability of samples (IC50) was 44.95 ± 2.52 μg/mL.
Table 2. *In vitro* antioxidant capacity of SF.

<table>
<thead>
<tr>
<th>Assay</th>
<th>TPC Assay</th>
<th>FRAP Assay</th>
<th>ABTS Assay</th>
<th>DPPH Assay</th>
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<tbody>
<tr>
<td></td>
<td>mg /g</td>
<td>TAC (µmol/g)</td>
<td>TAC (µmol/g)</td>
<td>IC50 (µg/mL)</td>
</tr>
<tr>
<td>SF</td>
<td>25.50 ± 0.73</td>
<td>17.16 ± 0.42</td>
<td>30.16 ± 0.12</td>
<td>44.95 ± 2.52</td>
</tr>
</tbody>
</table>

Total antioxidant activity of SF is expressed as ascorbic acid equivalents (µmol/g of dry extract). Total phenolic content (TPC) is expressed as gallic acid equivalents (mg/g of dry extract). Values are means ± SME of three experiments.

### α-Glucosidase inhibitory activity of SF

The α-glucosidase inhibitory activity of SF was depicted in Figure 3. The result showed that the extracts inhibited α-glucosidase activity dose-dependently (12.5–50 µg/mL). As was shown in Figure 3, the α-glucosidase inhibitory activities increased with the gradually increased concentration of SF. Based on IC50 values, α-glucosidase inhibitory activity of SF (IC50 = 167.4 µg/mL) was lower than that of acarbose standard reference (202.65 µg/mL).

![Figure 3. Dose-dependent changes in α-glucosidase inhibitory activity of SF (IC50 = 167.4 µg/mL of dried sample). Values are means ± SME of three experiments.](image)

### Standardizations of SF by HPLC

The HPLC profile of SF extract was run in parallel to the corresponding standards under similar analytical conditions. The HPLC analysis of rosmarinic acid and carnosol of rosemary crud extract are shown in Figure 4 and Table 3. HPLC analysis of SF extract revealed the presence of some chromatographic peaks among which are rosmarinic acid at 17.654 min and carnosol at 50.246 min. HPLC quantitative analysis showed that rosmarinic acid content was 3.0 mg per g SF crud extract while carnosol was 4.5 mg per g of SF crud extract.
Figure 4. Representative HPLC profile of SE ethanol extract at 280 nm (a), run in parallel to the rosmarinic acid and carnosol standards (b) under similar analytical conditions. Rosmarinic acid (peak 1), carnosol (peak2), Values are means ± S.E.M. of three experiments.

Table 3. The quantitative analyses of the SF ethanol extract carried out using an HPLC.

<table>
<thead>
<tr>
<th>SE extract</th>
<th>Peak No</th>
<th>Active ingredient</th>
<th>RT</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Rosmarinic acid</td>
<td>17.65</td>
<td>3.0mg/g</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Carnosol</td>
<td>50.25</td>
<td>4.5mg/g</td>
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</tr>
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</table>

Values are means ± S.E.M of three triplicate experiments.

DISCUSSION

The present study exhibited the protection effect of SF against STZ-induced oxidative stress in heart, kidney and liver of rats. The anti-oxidative stress of SF was confirmed by its antioxidants properties in vitro study. In the present study, we clearly showed that SF markedly decreased hyperglycemia in STZ-induced diabetic rats and restored the loss of body weight. In the present study, STZ administration caused an increase in the LP and decreases TAC in diabetic rats when compared to normal rats. MDA is the end product of LP and TAC considers the cumulative action of all the antioxidants present in the body. The increase in lipid peroxidation might be an indication of a decrease in enzymatic and non-enzymatic antioxidants of defense mechanisms. Oxidative stress is the common player for the major pathways associated with development and progression of diabetic complications. Oxidative stress damage was confirmed in the present STZ-treated rats by the increased in MDA level and the decreased in TAC in diabetic heart, kidney and liver, which is consistent with other studies. The increase in oxygen-free radicals in diabetic condition could be due to increase in blood glucose levels, which generate free radicals upon auto oxidation. In fact, increased generation of ROS such as superoxide, hydrogen peroxide and hydroxyl radicals have been shown to occur in diabetes in association with hyperglycemia. Oxidation, protein glycation and protein kinase activation are the main mechanisms of increased production of ROS in hyperglycemia and diabetes. One of the main challenges of research in recent years is to attenuate oxidative stress in order to improve diabetes. In our study, the treatment...
with SF completely prevented the elevation in MDA and the depletion in TAC induced in diabetic rats. This indicates that SF played a protective role in the diabetic tissues during oxidative damage. Cells possess a plenty of cytoprotective enzymes, which protect living cells from oxidative stress-induced damage. The antioxidant enzymes CAT and SOD are the first line of defense against free radical-induced tissue damage. The current work showed a significant increase in CAT and SOD enzyme activities in the heart, kidney and liver of diabetic rats except CAT activity showed a decrease in the diabetic liver. Diabetes was previously shown to increase CAT and SOD activities in heart and kidney of diabetic animals. This compensatory increase in CAT and SOD activities could be explained by the compensatory mechanisms in tissues to overcome the oxidative stress and may be response to increase production of hydrogen peroxide and superoxide radicals. In this line, Weidig et al. observed that increased generation of ROS in coronary microvascular endothelial cells under the effect of hyperglycemia increased the expression and activity of CAT, SOD and glutathione peroxidase enzymes. On the other hand, the decrease in hepatic CAT activity of diabetic rats, which previously observed may be due to oxidative stress induced inactivation and / or consumption of enzyme. The treatment with SF prevented these alterations in antioxidant enzymes in diabetic rats.

GSH serve as sensitive marker of oxidative stress and it plays an important role in maintaining the integrity of mitochondria and cell membranes. GSH content was significantly decreased in liver of diabetic rats, which was concomitant with previous studies. In contrast, GSH contents were significantly increased in heart and kidney of the same diabetic animals. This compensatory increase in GSH content in heart and kidney was previously reported in other studies. Because GSH is mainly synthesized in the liver, the decrease in hepatic GSH observed here could be resulted from increase its consumption by other tissues. In the present work, SF administration normalized GSH contents in heart, liver and kidney of diabetic rats. The antioxidant activity exhibited by SF could be one of the reasons for the protective effect of plant against oxidative stress in diabetic rats. The antioxidant potential of SF was also measured by scavenging activity on ABTS and DPPH radicals and reducing ability by FRAP assay. Present investigation showed that the SF exhibited significantly higher ABTS and DPPH scavenging activities, which demonstrates a potent hydrogen-donating ability of this plant. It has been established that salvia species and some of its constituents, mainly phenolic compounds such as salvianolic acids, rosmarinic acid, carnosol, cafeic acid and other phenolic glycosides have antioxidant effects in vivo and in vitro studies. However, the treatment with SF not only prevented the oxidative stress but also attenuated the hyperglycemic effects of diabetes. Several species of salvia including SF found to have anti-hyperglycemic effect in alloxan-induced diabetic animals. Hyperglycemia is not only generated more ROS but also attenuates antioxidative mechanisms through glycation of the antioxidant enzymes. So the possibility of anti-hyperglycemic effect of SF could not rule out for the protective effect offered in SF-treated diabetic rats. The possible mechanism by which SE brings about its anti-hyperglycemic action may be by inhibition of the breakdown of disaccharides to liberate glucose. Intestinal α-glucosidase catalyzes the breakdown of disaccharides to liberate glucose, which is later absorbed into the blood circulation. Inhibition of this enzyme would slow down the breakdown of starch in the gastro-intestinal tract, thus reducing postprandial hyperglycemia. SF extract exhibited remarkable inhibitory activities against α-Glucosidase (IC$_{50}$ = 167.4µg/mL).

**CONCLUSION**

Oxidative stress induced by diabetes could play a crucial role in the development and progression of diabetic complication and of SF could be a potential therapeutic agent for diabetic oxidative damage due to its antioxidant and anti-hyperglycemic properties.

**ACKNOWLEDGMENT**

There are no conflicts of interest regarding each of the authors involved in this research study.

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