Phytochemical and Pharmacological Screening for Antidiabetic Activity of Salvia aegyptiaca L. Ethanolic Leaves Extract

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Authors’ contributions

“This work was carried out in collaboration among all authors. Author FA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors AMAS and RAS managed the analyses of the study. Authors JMM and ASM managed the literature searches. All authors read and approved the final manuscript.

ABSTRACT

Diabetes mellitus is a common metabolic disorder of carbohydrate, fat, and protein, which results in high levels of glucose in the body after a meal or fasting. This disease is caused by the absence or reduction of insulin secretion. Accordingly, diabetes is usually classified into two types, Type 1(IDDM) and Type II (NIDDM). The aim of the present study is to carry the phytochemical analysis...
1. INTRODUCTION

Diabetes mellitus may be a widespread epidemic disorder caused by hormone (insulin) deficiency, diminished secretion, or each of them. This illness is one of the regular metabolic issue that influences 8.2% of the total population and is predicted to reach 4.5% in 2025. The incidence of DM (Diabetes Mellitus) is tremendous increasing particularly in developing countries. The illness causes substantial morbidity, mortality and long complications like retinopathy, neuropathy and nephrosis. It is found to harm many elements of the body system, notably the veins and nerves [1]. Numerous medicative plants are used for years in way of life to treat ailment everywhere throughout the world. As per WHO, medicative plants are the best source to get variety of natural medications. The use of plant extracts and phytochemicals, with known antioxidant, anti-diabetic & antihyperlipidemic properties may be of vast importance in therapeutic treatment [2,3]. Natural medicines, containing various active ingredients obtained as crude fractions, extracted from aerial and underground components of plant are widely utilized in healthcare or as dietary enhancements. One of the significant disadvantages of these medications is restricted bioavailability, being ineffectively ingested whenever taken orally [4-6].

More than 400 species of medicinal plants have anti-diabetic properties; however, the mechanism of their impact isn’t well outlined. Most medicinal herbs that have anti-diabetic effect are incorporate in the family of Asteraceae, Araliaceae, Cucurbitaceae, Leguminosae, Lamiaceae, Liliaceae, Moraceae and Rosaceae. The antidiabetic activities of medicative plants are accredited to the presence of terpenoids, polyphenols, flavonoids, coumarins and different constituents which show decline in blood glucose levels.

Plants used to treat diabetes often affect their effects by increasing insulin secretion, increasing glucose uptake by skeletal muscle and fat tissues, inhibiting liver glucose production and inhibiting intestinal retention of glucose. The main active components of diabetes are terpenoids, alkaloids, glycosides, steroids, carbohydrates, glycopeptides, amino acids and mineral ions. *Salvia* species has about 900 species in the world, including several medicinal, ornamental and culinary species. Approximately 58 species of Salvia have been known in Iran, including *S. mirzayanii*, *S. eremophila*, *S. macrosiphon* and *S. sahendica* as endemic species [7]. *Salvia* species are used throughout the world in traditional medicine and are active in terms of medicine, because of their insecticidal [8], antifungal, antioxidant [9], anti-diabetes, anticholinesterase and memory enhancement of patients with Alzheime’s disease [10]. *Salvia* species are rich sources of phenolic acids and flavonoids and quercetin. Flavonoids, in particular, have a positive effect on diabetes, by acting an inhibitory effect on the aldose reductase enzyme that may play a role in the complications of diabetes [11]. Quercetin reduces glucose and increases plasma insulin levels in STZ -induced diabetic rats [12]. Since glucose hemostasis is affected by several cases, in the current review, the hypoglycemic effects of *Salvia* species and molecular mechanisms of reduction blood glucose were studied separately.

A few degenerative issues, such as cardiovascular (CV) and brain diseases, diabetes, arthritis, cancer and immune system decline involve cellular harm probably associated...
to free radicals [13]. Antioxidants may scale back oxidative damages of biomolecules by modulating reactive free radicals. Thus, the need of antioxidants in food and cosmetics is clear [14].

However, natural residues of vital oils production ought to constitute a with ease to be had supply of useful compounds. Thus, wastes of the hydro-distillation process of fragrant plants oils had been studied for their contents of a diversity of biologically active compounds which includes antioxidants such as phenolic acids and flavonoids. Polyphenolics represent a normal group of secondary metabolites which can be largely represented in species of the genus Salvia. The primary part of these compounds are phenolic acids, mainly those based totally on caffeic acid constructing block, anthocyanins and as flavones, flavanols and their glycosides [15]. along with phenolic abietane diterpenes and their derivatives in Salvia plants [16]. It should be stated that post-distilled process of *Salvia Aegyptiaca* showed lower concentrations of phenolics in comparison to non-treated plants [17]. Differences discovered in composition of the plant extracts of distilled and non-distilled material ought to derive from the extraction situations carried out, including exposure to excessive temperature in the course of the hydro-distillation method. From the previous study, it is presumed that the crude acetone and methanolic extracts of *Salvia aegyptiaca* have Central nervous system (CNS) depressant effect, manifested as antinociception and sedation. Both extracts have been reported with few anti-inflammatory and antipyretic properties [18].

*Salvia aegyptiaca* L. belong to family Labiatae. In English, it is known as Egyptian sage and its Vernacular names are Shajarat al ghaazal and Ghabeisha. It is a green colour dwarf shrub which grows in varied locations within the Arabian Peninsula, Palestine, Egypt, Israel, Islamic republic of Iran and Afghanistan [19,20]. It is normally utilized as local folks ‘medicinal practices and in cosmetics. The seeds are usually used as a demulcent for diarrhoea and piles [21], while whole parts of plant are used in diarrhoea, gonorrhoea, hemorrhoids, eye diseases, antiseptic, antispasmodic and stomachic [22]. The plant is additionally utilized in neurological disorders, dizziness and trembling disorders [23].

Phytochemically, the entire plant consists of triterpenes, flavonoids, tannins, and coumarins [24]. Several flavonoids have been remoted from the plant, specifically, apigenin-7-glucoside, chrysoeriol-7-glucoside, luteolin 7-glucoside, 6,8-di-C-b-glucosyl luteolin and chrysoeriol-7-glucoside. Other parts isolated encompass lupeol, oleanolic acid, ursolic acid, b-amyrin, a-amyrin, and an essential oil. The roots had been reported to include diterpene quinones (aegyptinones A and B). Despite the vast phytochemical constituents on *Salvia aegyptiaca*, there very few reports on the pharmacological activities and no toxicological studies had been reported. In normoglycemic rats, the blood glucose concentration claimed to be decreased to half, when given at the dose of 0.25 g, after oral administration of extract of S. aegyptiaca [25]. Till date no work has been done on anti diabetic effect of *Salvia aegyptiaca* L. The aim of the present study to analyzed the phytochemical constituents and anti diabetic activity of the ethanol leaves extracts *Salvia aegyptiaca* L.

2. MATERIALS AND METHODS

2.1 Experimental Animals

Albino wistar rats’ weight about rodents 150grams to 200grams of both genders brought from Nizam Institute of Pharmacy, animal house located in Hyderabad. Before and at some stage in the experiment, rats were feeded with well-known diet. After randomization into different gatherings and before inception of investigation, the rats were acclimatized for a time of 7 days under standard ecological states of temperature, relative dampness, and dim/light cycle. Rats depicted as fasting were denied of food and water for 16 h ad libitum.

2.2 Acute Oral Toxicity Studies

*Salvia aegyptiaca* L. extract at the dose range of 100mg-2000mg/kg was administered orally to various group of rats comprising of 10 rats in each gathering. Mortality was seen after 72hours of administration. Acute toxicity studied were carried according to the procedure described by Litchfield and Wilcoxon [26].

2.3 Experimental Design

2.3.1 Treatment pattern in normoglycemic animals

Five groups of animals, 6 animals in each group received the below treatment pattern

Group I: Normal control
Group II: Standard Tolbutamide (50mg/kg, p.o)
Group III: Salvia aegyptiaca L (250mg/kg, p.o)
Group IV: Salvia aegyptiaca L (500mg/kg, p.o)

The above study is undertaken in two different animals' models. Wisters Rats of either sex weighing about 140-160 gms were selected for study. The animal was divided into four groups of six each and fastened overnight. The first group taken as control group was given carboxymethyl cellulose 2 gms, dissolved in 5 ml of water for 14 days. The second group was given the standard drug Tolbutamide 50 mg/kg for 14 days. The rest of the groups were given Salvia aegyptiaca L in dose of 250 mg/kg and 500 mg/kg for 14 days respectively. The blood was collected from retroorbital plexus on 1st day, 7th day and 14th day and glucose levels were estimated by glucometer.

2.3.2 Induction of diabetes in experimental animals

The experimental rats were made diabetic by a single dose intraperitoneal (i.p) infusion of alloxan monohydrate (100 mg/kg) [27]. Alloxan were weighed separately for every rat as per the body weight and afterward solubilized with 0.2 ml solution of saline (154mM NaCl) only before infusion. Two days after alloxan infusion, animals with plasma glucose levels of >140 mg/dl were considered for the examination. Treatment with plant extracts was began 48 hours after alloxan infusion.

2.3.3 Treatment pattern in diabetes animals

Five groups of animals, 6 animals in each group received the following treatment pattern

Group I: Normal control
Group II: Alloxan treated (100mg/kg,ip)
Group III: Alloxan (100mg/kg,i.p) + Standard Tolbutamide (50mg/kg, p.o)
Group IV: Alloxan (100mg/kg,i.p) + Salvia aegyptiaca L (250mg/kg, p.o).
Group V: Alloxan (100mg/kg,i.p) + Salvia aegyptiaca L (500mg/kg,p.o)

It consists of observing the effect of the Salvia aegyptiaca L in albino rats treated with alloxan induced diabetic rats. Wisters Rats of either sex weighing about 140-160gms were selected for study. All the animals were kept on the standard diet throughout the study, and were kept on fasting overnight before the study made next day. They were divided into five groups of six animal each. Salvia aegyptiaca L and standard drug Tolbutamide were administered with the assist of feeding cannula. Group I serve as normal control group, which received saline solution for 14 days. Group II to Group VI are diabetic rats (which previously received alloxan 100 mg/kg,ip) are given a fixed oral dose of Standard Tolbutamide 50 mg/kg, Salvia aegyptiaca L (250 mg/kg, per oral), and Salvia aegyptiaca L (500 mg/kg, per oral), for 14 consecutive days.

2.3.4 Collection of blood sample for blood glucose determination

Blood samples had been drawn from animal at weekly durations till the end of 2 weeks. Estimation of fasting blood glucose and determination of body weight were executed on day 1, 7, and 14 day of the study. Estimation of blood glucose may be accomplished by glucose test strips (electronic glucometer using).

2.3.5 Histopathology of pancreas of rats

The entire pancreas from each animal were removed after sacrificing the animal and was amassed in 10% solution of formaldehyde, and quickly processed by the paraffin method. Sections of 5µ thickness had been reduce and stained via hematoxylin and eosin (H & E) for histological examination. Histopathology of rat liver was done with the aid of a modified Luna method (Luna LG., 1999) [28]. In short, the autopsied livers have been washed in regular saline and glued in 10% formalin for 2 days accompanied with bovine solution for 6 hours. Then the rat’s livers have been paraffin embedded and fiveµ thickness microtone sections were made [29]. The sections have been processed in alcohol-xylene series and stained with hematoxylin and eosin. The histopathological slides were studied under a mild micro-scope for any histological damage/protection.

2.4 Statistical Analysis

All the estimations were communicated as Mean± S.E.M(standard error of mean) and examine for ANOVA and as well as Dunnett’s t-test.
3. RESULTS

The phytochemical screening of ethanolic leaves extract of *Salvia aegyptiaca* L shows the presence of phenols, flavonoids, steroids, proteins, glycosides, carbohydrates, lipids, alkaloids, tannins and terpenoids, but saponins are absent are shown in Table 1.

The acute oral toxicity study of *Salvia aegyptiaca* L ethanolic leaves extract showed no mortality upto 2000 mg/kg. Hence extract found to be safe for animal dosing. The effect of *Salvia aegyptiaca* L ethanolic leaves extract in normoglycemic animals are shown in the Table 2 and Graph 1. Group II which received standard Tolbutamide (50 mg/kg) alone for 14 days showed reduction in blood glucose level, while Group III and IV received *Salvia aegyptiaca* L (250 mg/ kg, p.o) + Alloxan (100 mg/kg.ip) and *Salvia aegyptiaca* L (500 mg/ kg, p.o)+ Alloxan (100 mg/kg.ip) for 14days also showed decrease in blood glucose level, indicating effectiveness of *Salvia aegyptiaca* L ethanolic leaves extract in treating diabetes. Histopathology of rats pancreas shows no damage to islet cells of pancreas in alloxan treated groups, indicating antidiabetic activity of *Salvia aegyptiaca* L (Figs. 1-5).

<table>
<thead>
<tr>
<th>Phytochemical compounds</th>
<th>Present/Absent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenols</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>+</td>
</tr>
<tr>
<td>Proteins</td>
<td>+</td>
</tr>
<tr>
<td>Glycosides</td>
<td>+</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>+</td>
</tr>
<tr>
<td>Lipids</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>-</td>
</tr>
</tbody>
</table>

+= Present  -=Absent

Table 2. Effect of *Salvia aegyptiaca* L. on basal blood glucose levels in normoglycemic rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Blood glucose level (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Day 1</td>
</tr>
<tr>
<td>Groups I</td>
<td>Normal control</td>
<td>83.2±2.4</td>
</tr>
<tr>
<td>Groups II</td>
<td>Standard Tolbutamide (50mg/kg)</td>
<td>84.6±8.1</td>
</tr>
<tr>
<td>Groups III</td>
<td>Salvia aegyptiaca L (250mg/ kg, p.o)</td>
<td>86.4±6.3</td>
</tr>
<tr>
<td>Groups IV</td>
<td>Salvia aegyptiaca L (500mg/ kg,p.o)</td>
<td>81.1±3.8</td>
</tr>
</tbody>
</table>

Values are mean ± SEM (n=6) one-way ANOVA followed by Dunnett’s test, Where, * represents significant at <0.05, ** represents significant at < 0.01, and *** represents very significant at p<0.001. All values are compared with normal control

Table 3. Effect of *Salvia aegyptiaca* L. on blood glucose in alloxan treated rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Blood glucose level (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Day 1</td>
</tr>
<tr>
<td>Groups I</td>
<td>Normal control</td>
<td>81.4±1.4</td>
</tr>
<tr>
<td>Groups II</td>
<td>Alloxan (100mg/kg,i.p)</td>
<td>212.4±3.4</td>
</tr>
<tr>
<td>Groups III</td>
<td>Alloxan (100mg/kg,i.p) + Standard Tolbutamide (50mg/kg,p.o)</td>
<td>210.2±5.2</td>
</tr>
<tr>
<td>Groups IV</td>
<td>Alloxan (100mg/kg,i.p) + <em>Salvia aegyptiaca</em> L (250mg/ kg,p.o)</td>
<td>215.3±2.4</td>
</tr>
<tr>
<td>Groups V</td>
<td>Alloxan (100mg/kg,i.p) + <em>Salvia aegyptiaca</em> L (500mg/kg, p.o)</td>
<td>220.6±2.5</td>
</tr>
</tbody>
</table>

Values are mean ± SEM (n=6) one-way ANOVA followed by Dunnett’s test, Where, * represents significant at <0.05, ** represents significant at < 0.01, and *** represents very significant at p<0.001. All values are compared with toxicant.
Graph 1. Effect of *Salvia aegyptiaca* L. on basal blood glucose levels in normoglycemic rats

Graph 2. Effect of *Salvia aegyptiaca* L. on blood glucose in alloxan treated rats

3.1 Histopathology of Diabetic Rats Pancreas

Fig. 1. Group I-Shows normal pancreas

Fig. 2. Group II-Shows decrease number of Islet and damage of Acini
shows the presence of many compounds such as, phenols, flavonoids, steroids, proteins, glycosides, carbohydrates, lipids, alkaloids, tannins and terpenoids, but saponins are reported to be absent. The acute toxicity of ethanolic leaves extract of \textit{Salvia aegyptiaca} L were tested, its shows that high doses of extract were tolerated without significant over mortality or signs of toxicity. The relatively high dose of the extract used in the current pharmacological experiments may represent the low concentration of the active constituents present in the extract.

In the previous study, it has reported that crude extract methanol and acetone of \textit{S. aegyptiaca} have central nervous system (CNS) depressant effect, manifested as sedation and antinociception. Both extracts had also reported with anti-inflammatory and antipyretic activity [30]. It has previously reported that atropine inhibits the hypotensive and spasmogogenic actions of \textit{Salvia aegyptiaca} [31].

The recent study also reported that phytochemical screening of \textit{Salvia} species shows the presence of many compounds belonging mainly to the group of phenolic acids, coumarins, polysaccharides, phenolic glycosides, flavonoids, sterols, terpenoids, anthocyanins and essential oils [32]. Numerous mechanisms of actions have been suggested for \textit{Salvia} species. A few theories identify with their impacts on the movement of pancreatic beta cells, increment in the inhibitory impact against the insulinase protein, increment insulin affectability or the insulin like impact of the plant extracts. Different numerous mechanisms additionally be included, for example, enhanced peripheral usage of glucose, expanding the synthesis of hepatic glycogen or decline of glycogenolysis, hindrance of intestinal retention of glucose, decrease in glycemic index of starches and decrease in the impact of glutathione.

It has reported that methanol extracts of \textit{Salvia aegyptiaca} showed a phenolic description, composed of 14 recognize phenolic compounds. Polyphenolics of \textit{Salvia aegyptiaca} composed of 4 phenolic acids (ferulic acid, p-hydroxybenzoic acid, caffeic acid, and rosmarinic acid), 3 phenolic diterpenes (carnosol, methyl carnosate, carnosic acid), 5 flavones (cirsimaritin, apigenin, genkwanin, luteolin and salvigenin) and 2 flavone glycosides (luteolin-7-Oglucoside and apigenin-7-glucoside). Many of the Salvia species conformed to be rich in phenolic compounds,
specially; rosmarinic acid was highly enormously represented in the genus [33-36]. A huge spectrum of natural activities including antioxidant, anti-inflammatory, antiviral activities, antitumor and antibacterial were related to the bioactive compounds of Salvia species, for example, predominantly polyphenolics. Previously it was defined that good antioxidant activity was linked to the highest amount of rosmarinic acid. It is also stated that numerous biological effects of rosmarinic acid including hinder the HIV-1, preventing the blood clots, antitumor, anti-inflammation, antihepatitis and securing the liver. Data published within the literature confirmed that the influence of the distillation method on polyphenolic contents in several species Salvia of the Lamiaceae family.

5. CONCLUSION

In this research article phytochemical and antidiabetic activity of Salvia aegyptiaca L extract were studied. The phytochemical screening of Salvia aegyptiaca L ethanolic leave extracts showed the various phytochemical constituents. This study also showed that the leaves extracts of Salvia aegyptiaca L (250 mg/kg and 500 mg/kg) significantly decreases the glucose level in both normoglycemic and alloxan induced diabetics rats, when compared with standard Tolbutamide group. Previous literature had reported that, polyphenolics are the important phytochemical responsible for various pharmacological properties and as well as antioxidant activities. Further research is expected to decide the specific mechanism(s) of activity of the different constituents in the extract concentrates. It is conceivable that the extract contains numerous compound that exerts more than one activity by means of various mechanisms.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Animal ethic committee approval has been collected and preserved by the author all animal analyse was done as per the rules of cpcsea and research study was endorsed by the iaec (institutional animal ethical committee) nizam institute of pharmacy, hyderabad, india, with registration number (1330/ac/10/cpcsea).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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