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Research Article
Effect of the Methanolic Extract of Algerian *Salvia officinalis* L. Against Oxidative Stress Associated with Diabetes Mellitus

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Abstract

Background and Objective: Medicinal plants are considered an enormous source of multiple phytotherapeutic substances used to treat various human diseases. Hence, the present study aims to evaluate the effect of the methanolic extract of *Salvia officinalis* L. against oxidative stress in streptozotocin-diabetic rats. **Materials and Methods:** Methanolic extract was prepared on the differential solubility of polyphenolic compounds in organic solvents. Diabetes was experimentally induced in rats by streptozotocin (40 mg kg^{-1}). The treated groups received, 300 mg kg^{-1} of methanolic extract and gallic acid. The weight and blood glucose levels were controlled from the beginning of the treatment. In terms of experimentation, the liver was removed and a cytosolic dosage of catalase, superoxide dismutase, glutathione and malondialdehyde were performed to estimate possible oxidative damage caused by the installation of diabetes. **Results:** The method of extraction adopted yielded a methanolic extract with a percentage of 10.63%. The determination of polyphenols revealed a content of 10.43±1.01 mg g^{-1} EAG/mg of extract and that of flavonoids of 2.26±1.42 mg g^{-1} EQ/mg of extract. Oral administration of the methanolic extract of *Salvia officinalis* L. lowered blood glucose and MDA and increased GSH, catalase and superoxide dismutase activities. **Conclusion:** These results suggested an important antioxidant effect of *Salvia officinalis* L., which can reduce oxidative stress and delay the onset of diabetes complications.

Key words: *Salvia officinalis*, polyphenols, diabetes, oxidative stress, streptozotocin, oxidative stress parameter

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Diabetes mellitus is a disorder/chronic metabolic syndrome resulting from a variety of interactions between hereditary and environmental factors[1]. It is characterized by hyperglycemia resulting from a lack of secretion, insulin action or both associated anomalies. There is growing evidence that diabetes complications are associated with oxidative stress[2]. Indeed, chronic hyperglycemia causes an imbalance between the production of unstable oxygen derivatives (free radicals) and/or insufficient neutralization by antioxidants (vitamins, trace elements and enzymes)[3]. Indeed, superoxide dismutase, catalase and glutathione peroxidase (antioxidant enzymes) can catalyze the neutralization of free radicals[4]. The activity of ROS can induce lipid peroxidation, which subsequently stimulates protein glycation, the inactivation of enzymes and the alteration of the structure and function of biomembranes, thus playing a crucial role in diabetes complications[5]. Given the dramatic increase in diabetes and the side effects of antidiabetic drugs, many researchers evaluated the pharmacological action of traditional plants and thus their interest in traditional medicine[6]. In addition to primary metabolites, plants have secondary metabolites which represent a major source of molecules that can be used by humans, particularly in the pharmacological field[7]. Among these interesting substances, polyphenols are an important class of secondary compounds[8]. *Salvia officinalis* L. commonly called Officinal Sage and part of the family Lamiaceae (labiatae), is a Euro-Mediterranean plant, widely used in a traditional way to treat diabetes in Algeria. The aim of this work was to study the antidiabetic and antioxidant activity of polyphenolic extract of the leaves of the *Salvia officinalis* plant by performing a series of tests on Wistar rats made diabetic with streptozotocin as a diabetes model.

MATERIALS AND METHODS

Study area: The current study was carried out from March to July, 2019 at the University M'hamad Bougara of Boumerdes, Algeria.

Plant material and identification: Experiments were carried out on the leaves of *Salvia officinalis* commonly cultivated in different areas of Algeria. The plant was identified by Doctor Abdekrim, Taxonomist Botany in the Department of the National Superior School of Agronomy (ENSA). Leaves were obtained by handpick and collected in March, 2019 near Khemis El-Khechna, Boumerdes, Algeria. Leaves were allowed to air dry at room temperature and then ground using a microwave (waring). Dried leaves were powdered (100 g) and stored in a dry and dark place.

Extraction procedure: The methanolic extract of the leaves of *Salvia officinalis* (MESO) was prepared according to the method of Tadeg and collaborators[9]. Plant powder is macerated in a methanol/water mixture (8: 2 V/V) at a ratio of 10 g/100 mL under gentle agitation for 48 hrs at room temperature. The hydroalcoholic extract is recovered after filtration of the mixture. The methanol is then removed from the filtrate by evaporation under reduced pressure at 50°C. The percentage of crude dry extract was determined as follows[9]:

$$\text{Y extract (\%)} = \frac{\text{M extract}}{\text{m feed}} \times 100$$

Dosage of total polyphenols: The polyphenol content is estimated by the Folin-Ciocalteu method[10]. As 1 mL of the methanolic extract was mixed with 1mL of Folin-Ciocalteu (2 M) diluted 10 times and 1 ml L^{-1} of Sodium Carbonate (Na_2CO_3) at a concentration of 75 g L^{-1}. Absorbance is measured at 765 nm, after incubation for 1 hr at room temperature (spectrophotometer type UV Optizen 2120, Korea). The calibration curve is carried out with gallic acid, following the same steps of the assay. Total phenolics content was expressed as µg of gallic acid equivalent/g of dry extract (µg GAE/g).

Determination of total flavonoid content: The quantitative evaluation of flavonoids was performed using the colorimetric method of aluminum trichloride[11]. As 1 mL of the extract with the appropriate dilutions is added to an equal volume of a 2% $AlCl_3$ solution. After 10 min of incubation, the mixture is vigorously stirred and the absorbance was taken at 430 nm. The concentration of flavonoids in percentage expressed as equivalents of quercetin milligrams per gram of sample weight (mg EQ/g E).

Qualitative analysis by High-Performance Liquid Chromatography (HPLC): High-Performance Liquid Chromatography (HPLC) is performed using an Agilent 1100 Liquid Chromatograph. The stationary phase used is Hypersil BDS-C18 (5 µm and 250×4.6 mm) thermostated at 3°C. The mobile phase consists of water acidified with acetic acid (at pH 3.1) and acetonitrile. The pump flow rate is

set at 1 mL min^{-1} throughout the analysis. An autosampler temperature-controlled (automatic sampler AS 100) is used. The obtained data are processed by the UV chromera flexar software (version 4.1.0) for the analysis and integration of peaks. The polyphenols were identified by comparing their retention times relative to the corresponding standards, quercetin, rutin, gallic acid, luteolin glucuronide, rosmarinic acid, apigenin glucuronide, luteolin acetyl glucuronide and hispidulin glucuronide.

Scavenger effect of the radical DPPH: The anti-free radical activity of the different extracts is determined by the method using DPPH (2,2-Diphenyl-1-Picrylhydrazyl) as a relatively stable free radical[12]. The DPPH solution was prepared by dissolving 2.4 mg of DPPH in 100 mL of methanol. The MESO at different concentrations was prepared in absolute methanol. The 25 µL of the different solutions tested are added to 975 µL of the DPPH solution. After shaking, the tubes are placed in the dark for 30 min. The reading is carried out by a spectrophotometer at 517 nm (spectrophotometer type UV Optizen 2120, Korea). The anti-radical activity or the antioxidant power was estimated according to the equation:

$$(A\%) = \frac{A0 - At}{A0} \times 100$$

Where, A% is percentage of the anti-radical activity, A0 is absorbance of the negative control and At is absorbance of the sample.

Experimental animals: Adult male Wistar rats, weighing 120-200 g, purchased from the Pasteur Institute of Algeria. They were treated in accordance with the criteria set out in the "Guide for the care and use of animals" prepared by the National Academy of Sciences and published by the National Institute of Health. Rats were carried in plastic cages with stainless steel cover. The experiment was conducted at room temperature (20°C) with a natural cycle of light and darkness. Water and food were provided *ad libitum*.

Induction of experimental diabetes: Induction of experimental diabetes was performed by an intraperitoneal injection of 40 mg kg^{-1} of streptozotocin freshly prepared in a cold solution of 0.1 M citrate buffer (pH 4.5). The 1 week after the injection, the blood glucose levels of 20 rats were taken using a glucometer (Acon plus type) and animals whose blood glucose was greater than 180 mg dL^{-1} were considered diabetic[13].

Study of the *in vivo* antioxidant activity

Preventive effect of methanolic extract on experimental diabetes: To study the *in vivo* antioxidant activity, 20 rats divided into four batches are used: Group 1 (TMESO), received before and after 2 hrs of injection of streptozotocin, 300 mg kg^{-1} of MESO. Group 2 (TAG) received before and after 2 hrs of injection of streptozotocin, 300 mg kg^{-1} of gallic acid. Group 3 (DT) received, before and after 2 hrs of injection of streptozotocin, 1 mL of the NaCl solution. Group 4 (TS), received nothing. The evolution of the glycemia of each batch was evaluated using a glucometer before and after 5 days of treatment, then the rats were sacrificed by cervical dislocation and the livers were collected[5].

Evaluation of the parameters of the redox status

Determination of lipid peroxidation: For the determination of MDA, 0.5 g of liver was added to 3 mL of phosphate buffer solution (0.1 M and pH 7.4) containing KCl (1.15 M). This mixture was milled at 1200 rpm (HS 30D DAIHAN Scientific Co., Ltd., Korea). To 0.5 mL of the recovered homogenate, 0.5 mL of trichloroacetic acid (20%) and 1 mL of thiobarbituric acid (TBA) (0.67%) (Sigma Aldrich T5500, Germany) are added. The mixture is heated at 100°C for 15 min, cooled and then added with 4 mL of n-butanol. The concentration of MDA was deduced from a standard range with 1, 1, 3, 3-tetraetoxypropane[14].

Determination of hepatic reduced Glutathione (GSH): To assay the GSH, 0.3 g of liver was homogenized in 3 volumes of 5% trichloroacetic acid (TCA) and then centrifuged at 2000 rpm for 5 min. To 10 mL of phosphate buffer (0.1 M, pH 8), 50 µL of supernatant was diluted. The 20 µL of DTNB (0.01 M) were added to 3 mL of the dilution mixture. The reading of the optical density was carried out after 5 min at 412 nm[15]. The GSH concentrations were deduced from a standard range established with pure glutathione under the same conditions.

Determination of enzymatic antioxidant defenses: For the determination of antioxidant enzymes and in order to prepare an enzymatic fraction, 2 g of liver are cut and homogenized in 3 volumes of phosphate buffer (0.1 M, pH 7.4) containing KCl (1.17%)[16]. The homogenate is centrifuged at 2000 rpm for 15 min at 4°C. The supernatant obtained was centrifuged at 9600 rpm for 30 min at 4°C and the final supernatant.

Evaluation of enzymatic activity of superoxide dismutase (SOD): For the determination of SOD, a reagent medium consisting of 300 µL of the phosphate buffer (0.1 M and pH 7.4) of 300 µL of a solution of NTB at 45 µmol of 300 µL of 20 µmol sodium cyanide of 300 µmol of methionine at 45 µM of 300 µL of riboflavin at 2 µmol and 300 µL of cytosol. This mixture was exposed to the light of a 15 watt lamp for 10 min to induce photoreaction of riboflavin and O_2. The reduction of NBT by the formazan superoxide anions was followed by the (spectrophotometer-type UV Optizen 2120, Korea) at 560 nm[17]. The enzymatic activity was calculated in terms of IU mg^{-1} of protein according to the equation:

$$\text{Total inhibition} = \frac{\text{ODW} - \text{ODS}}{\text{ODW}} \times 100$$

Where, ODW is optical density of white and ODS is optical density of sample.

One unit of SOD activity is defined as the enzyme that would cause 50% inhibition of superoxide anions:

$$\text{SOD unit} = \frac{\text{Total inhibition}}{n \times 50}$$

Where, n is mg of protein present in the volume of the sample used.

Protein assay: Protein concentrations in the cytosol supernatants of liver homogenates were determined by the Bradford method[18]. A standard range of 0.1 to 0.8 mg mL^{-1} was made from different dilutions of a 0.1% solution of BSA (Sigma-Aldrich). The optical density was measured spectrophotometrically at 595 nm. The protein concentration was expressed in mg mL^{-1}.

Evaluation of catalase enzymatic activity (CAT): For the CAT assay, 1 mL of phosphate buffer (0.1 M KH_2PO_4, pH 7.2), 0.975 mL of H_2O_2 (0.091 M) and 0.025 mL of the cytosolic fraction were mixed. The reaction was monitored by continuous reading of the absorbance change at 240 nm every 30 sec for 2 min[19]. The activity of the enzyme was expressed in Units/mg of liver tissue protein, according to the formula:

$$\text{UI / mg} = \frac{2.3033}{T} \times \log \frac{A1}{A2}$$

Where, A1 is absorbance at the 1st min, A2 is absorbance at the 2nd min and T is time interval in min.

Statistical analysis: The results were expressed as Mean±Standard error at mean (Mean±SEM) of 5 rats per group. Statistical analysis was performed using Statistica software (version 6, Genistat Conseils Inc., Montreal). After analysis of variance, the comparison of averages was performed by the student's t-test for paired samples. At a 95% confidence interval, a $p \leq 0.05$ value was considered statistically significant.

RESULTS

Determination of yield of extraction and total flavonoids and phenols content: The extraction of polyphenols from the sage leaf powder yielded a methanol extract with a percentage of about 10.63%. The total phenolics content of MESO was 10.43±1.01 mg g^{-1} of gallic acid equivalent of plant extract with reference to a gallic acid standard curve ($y = 0.412x+0.0378$ and $R^2 = 0.9882$). The total flavonoid content of MESO was 2.26±1.42 mg g^{-1} of quercetin equivalent of plant extract with reference to quercetin standard curve ($y = 11.384x+0.1036$ and $R^2 = 0.9938$).

HPLC analysis: The 5 compounds were identified in the methanolic extract by comparing their retention times with those of the standards. Figure 1 shows the chromatograms of the plant extract. The HPLC profiles show the compounds mentioned by their peaks and retention time in the spectra of the extract studied. The detailed results were shown in Table 1.

Analysis of the methanolic extract by HPLC revealed a chromatogram represented by 5 peaks with retention times between 19.34 and 26.302 min. The composition of the extract shows that there were 5 polyphenolic components at different percentages: Luteolin-7-O-glucuronide (29.35%), rosmarinic acid (24.19%), apigenin 7-o-glucuronide (4.84%), Lutéoline 3'-o-glucuronide (4.19%), Hispidulin 7-glucuronide (3.55%) and 33.88% were unknown. The results showed that *Salvia officinalis* L. contains rosmarinic acid and its derivatives and flavonols, apigenin, luteolin and their derivatives.

Table 1: Retention time of polyphenols present in MESO

Compound	Rt (min)
Luteolin glucuronide	19.340
Rosmarinic acid	23.867
Apigenin glucuronide	24.304
Luteolin acetyl glucuronide	25.996
Hispidulin glucuronide	26.302

Rt: Retention time

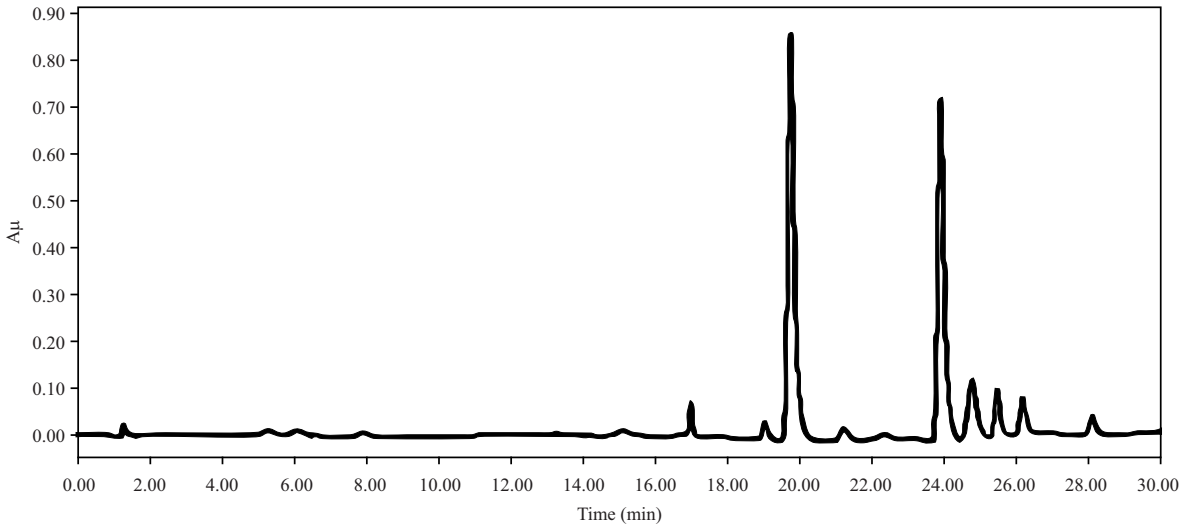

Fig. 1: HPLC chromatogram of the methanolic extract of *Salvia officinalis*

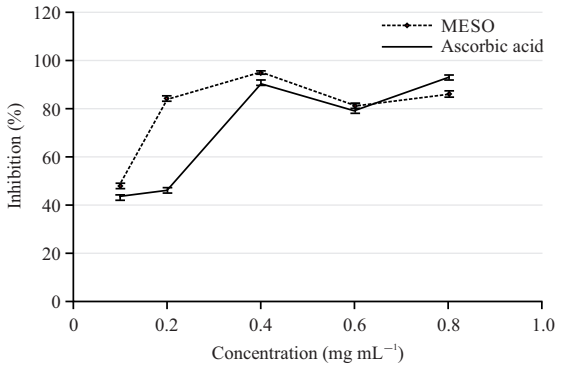

Fig. 2: Anti-radical power of MESO and ascorbic acid
Ascorbic acid is used for the positive control and each point represents the Mean±SEM (average of 3 tests)

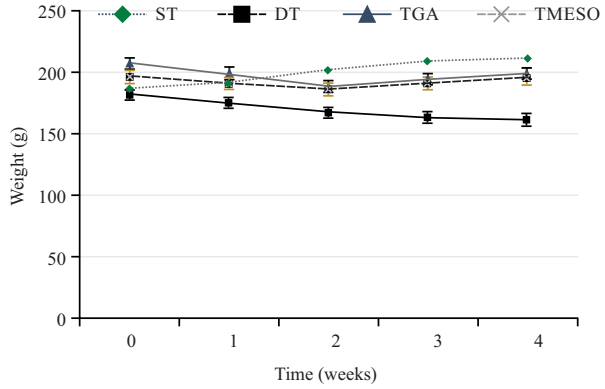

Fig. 3: Evolution of the weight of diabetic rats treated during the 5 weeks of treatment
Values are given as Mean±SEM (n = 5), student's t-test: ***p<0.001 and the difference is very highly significant

Evaluation of the anti-radical power of MESO: The results of the percentage inhibition of the free radical DPPH by MESO and ascorbic acid show an increase in the inhibition of the DPPH radical proportional to the concentrations of the phenolic extract. Indeed, the inhibition of this radical by the MESO achieved its maximum value (94.87±0.74%) comparable to that of ascorbic acid (90.74±1.13%) ($p<0.05$) (Fig. 2).

The profiles of the antiradical activity obtained revealed that the methanolic extract of *S. officinalis* as well as ascorbic acid have a dose-dependent activity. The results of the IC50 values for DPPH assay showed that the crude methanolic extract has a better antiradical power compared to that of ascorbic acid with respective values of 0.12±0.52 and 0.22±0.78 ($p\leq0.05$).

Preventive effect of methanolic extract against streptozotocin-induced diabetes

Evolution of the weight of the rats: The injection of streptozotocin-induced diabetes was characterized by loss of body weight. This decrease was of 4.17, 8.35, 10.71 and 11.70% ($p<0.001$) in the group of rats DT (Fig. 3). Furthermore, during the same periods, the TS group experienced a steady increase of 2.56, 7.97, 13.31 and 13.32% ($p<0.001$). However, the treatment with MESO has allowed an increase of 2.64 and 4.79% ($p<0.001$) after the third and fourth week of treatment, respectively. The same effect was observed in the TAG group with weight increase percentages of 1.24 and 3%, respectively.

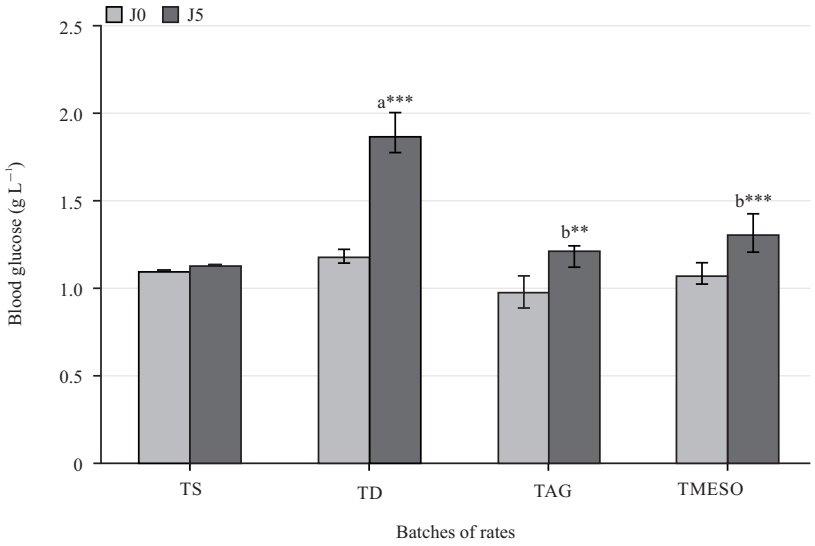

Fig. 4: Variation in blood glucose levels in rats pretreated or not with polyphenols
Values are given as Mean±SEM (n = 5), (a) Comparison with the same group before injection of streptozotocin and (b) Comparison with rats made diabetic by streptozotocin, student's t-test: **p<0.01 difference is highly significant and ***p<0.001 the difference is very highly significant

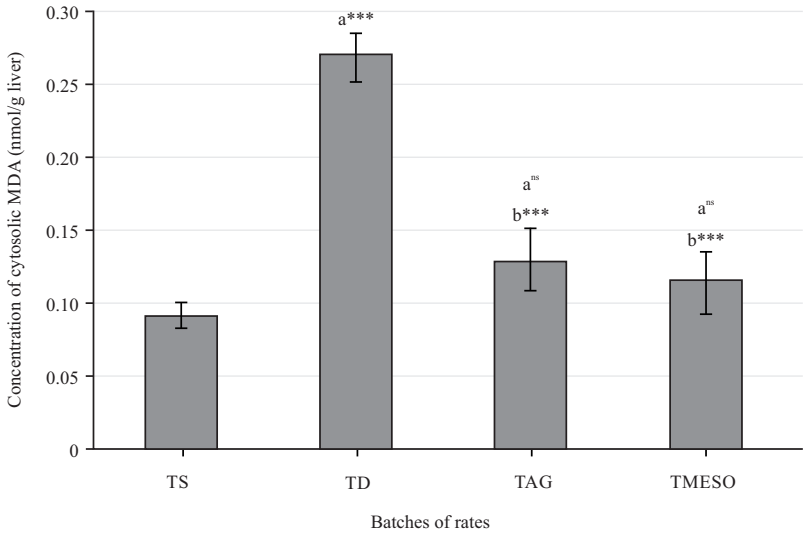

Fig. 5: Effect of polyphenols of *Salvia officinalis* L. on the production of MDA in liver cells in different batches of rats
Values are given as Mean±SEM (n = 5), (a) Comparison with normal rats and (b) comparison with rats made diabetic with streptozotocin, student's t-test: ns: Difference is not significant, **p<0.01 the difference is highly significant and ***p<0.001 the difference is very highly significant

Evolution of blood glucose: The results of the protective effect of MESO extract were illustrated in Fig. 4. In control diabetic rats, a very highly significant hyperglycemia compared to their initial blood glucose was found. This increase was in the order of 35.08% (p<0.01). However, a decrease of 15.32% in blood glucose was observed at day 5 in MESO-protected animals compared to NaCl-treated diabetic rats (p<0.001). A comparable effect was obtained in rats protected by gallic acid with a reduction percentage of the order of 21.17% (p<0.001).

Analysis of oxidative stress parameters: Figure 5 represented the variation of hepatic MDA levels in healthy rats and made diabetic by streptozotocin pretreated or not by polyphenols.

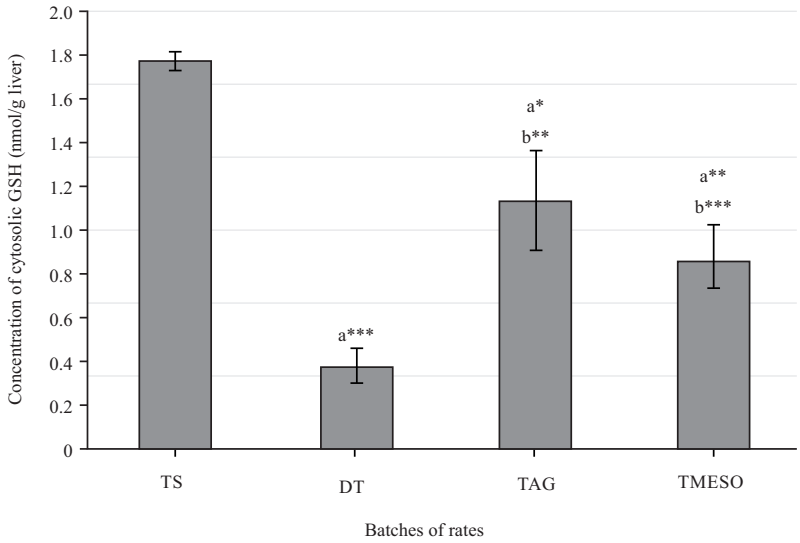

Fig. 6: Influence of the effect of polyphenols of *Salvia officinalis* on hepatic GSH concentration

Values are given as Mean±SEM (n = 5), (a) Comparison with normal rats and (b) Comparison with rats made diabetic with streptozotocin, student's t-test: *0.05>p>0.01, the difference is significant, **p<0.01 the difference is highly significant and ***p<0.001 the difference is very highly significant

Table 2: Effect of polyphenols of *S. officinalis* on the activity of CAT and SOD in diabetic rats

	Enzymes		
Batches	Proteins mg mL^{-1}	CAT activity (IU mg^{-1} proteins)	SOD activity (IU mg^{-1} proteins)
TS	6.45±0.1	2.68±0.2	18.30±0.015
DT	2.76±0.02*	1.14±0.02**	5.79±0.15***
TGA	4.78±0.01^{ns}	1.75±0.03***	11.13±0.77***
TMESO	4.21±0.19*	1.55±0.32***	13.01±0.14**

Values are given as Mean±SEM (n = 5), student's t-test: ns: Difference is not significant, *0.05>p>0.01, the difference is significant, **p<0.01 the difference is highly significant and ***p<0.001 the difference is very highly significant

A highly significant (p<0.001) increase in hepatic MDA in control diabetic rats compared to healthy control rats was found. This increase was of the order of 93.45%. However, a pretreatment with MESO decreased the hepatic MDA level by 42.66% in diabetic rats compared to TD (p<0.001). The same effect was observed for the treatment of diabetic rats with the standard antioxidant, quercetin, with a 44.73% decrease percentage (0.05>p>0.001).

Variation in hepatic Glutathione (GSH) reduced levels: The results of the effect of MESO on cytosolic rate variation in GSH were illustrated in Fig. 6. A very highly significant reduction in hepatic GSH level was observed in control diabetic (DT) rats compared to that recorded in healthy rats (ST). This decrease was in the order of 72.78% (p<0.001). However, pretreatment with MESO at a dose of 300 mg kg^{-1} increased the reduced GSH level by 49.14% compared to diabetic rats (0.05>p>0.001). These results remain lower compared to rats pretreated with gallic acid which increased the hepatic glutathione level by 54.78% (p<0.01).

Effect of polyphenols *S. officinalis* on the activity of catalase and superoxide dismutase: The results of the MESO effect on enzymes were reported in the Table 2.

A very highly significant reduction in the activity of CAT and SOD cytosolic was found. This reduction was of 45.19% and 70.36%, respectively (p<0.001). However, pretreatment with MESO caused a very highly significant increase of CAT activity (p<0.001) compared to diabetic temoins rats that received only the physiological solution.

DISCUSSION

The method of extraction adopted was used to extract the maximum amount of compounds and prevent their denaturation likely due to the high temperatures used in other extraction methods. In the current work, the yield obtained was10.63%. These results were similar to those already reported in the literature with solvents of the same density or with the use of other extraction methods[20]. It should be noted that the extraction yield is only relative and depends on the

geographical origin of the plant and the method and conditions under which the extraction was carried out[20].

It was interesting to determine the amount of flavonoids and polyphenols in the extract of *S. officinalis* to better characterize them. According to the results obtained, the concentration of polyphenols was 10.43±1.01 mg EAG/g of extract of the plant. In fact, the content of the total phenols is not stable and differs from one plant to another and between species of the same genus. These analyzes were similar on average to those found by some authors with values between 1.4-5.7 mg EQ/g extract for different species of sage[21].

For HPLC, the column used to separate the polyphenols was exclusively reverse phase. This system is a high-standard chromatographic resolution technique for the simultaneous separation and quantification of phenolic substances[22]. The characterization of MESO extract identified the presence of luteolin-7-O-glucuronide, rosmarinic acid, apigenin 7-o-glucuronide, lutéolin 3'-o-glucuronide and hispidulin 7-glucuronide. Current results were in agreement with some studies that confirmed that Sage species were characterized by polyphenolic compounds that have strong antioxidant activity[23]. The results of another study where the authors worked on 9 species of sage, ascended the presence of polyphenol carboxylic acids (rosmarinic acid, chlorogenic, caffeic acid) and flavonoids (Luteolin, apigenin luteolin-7-O-glycosides, apigenin-7-O- glycosides)[24].

The DPPH test performed revealed that the methanolic extract of *S. officinalis* has a remarkable activity with respect to the capture of DPPH comparable to ascorbic acid. The comparative study with species belonging to the same family of Lamiaceae shows differences in IC50 values. For example: *Teucrium polium* (IC 50 = 0.0201 mg mL^{-1})[25], *Salvia pisidica* (IC 50 = 4.8 mg mL^{-1})[26], *Nepeta flavida* (IC 50 = 0.0632 mg mL^{-1})[27]. This difference could be explained by the fact that the reaction between the antioxidant and the DPPH depends on the structural conformation of the antioxidant. Some compounds react rapidly with DPPH reducing the number of DPPH molecules equal to the number of hydroxyl groups[28]. The results obtained indicated the existence of a remarkable and significant linear correlation between the antiradical power of MESO and its content of phenolic compounds ($r^2 = 0.98$ and $p<0.01$).

Streptozotocin is a nitrosuric derivative that is one of several substances used to induce insulin-dependent or non-insulin-dependent diabetes in rats[29]. This drug causes necrosis of pancreatic β cells and severe insulin deficiency with diabetic hyperglycemia established in the following two days[30]. In this study, it was found that injection of STZ at a dose of 40 mg kg^{-1} could induce in rats the development of type 1 diabetes after 2 days of observation. The STZ-induced diabetes characterized by severe loss of body weight can lead to several complications related to diabetes. Body weight loss in STZ-diabetic rats is observed in our study where the DT group decreased after 5 days of follow-up. Body weight loss in STZ-diabetic rats was observed in this study where the DT group decreased after 5 days of follow-up. Current study results were in agreement with those reported by Pari and Latha[31], who found significant body weight loss in streptozotocin-diabetic rats. Treatment of rats with MESO and gallic acid improved the change in body weight relative to the DT group. The ability of the extract to protect diabetic rats from massive body weight loss appears to be due to its ability to reduce lipid levels and its hypoglycemic effect through improved insulin secretion[32].

The STZ-induced hyperglycemia causes excessive formation of ROS by various pathways such as glucose autoxidation, the polyol pathway and protein glycation[33]. In this study, after 5 days of STZ administration to rats, hyperglycemia has set in. This effect was explained by the fact that the STZ is provided with a hydrogen peroxide producing power and other free radicals which are at the origin of this cellular necrosis[34]. However, rats pretreated with polyphenols are protected against the deleterious and diabetogenic effect of streptozotocin. As a result, a drop in blood glucose is noted on the fifth day in rats protected by MESO and gallic acid. These results were in agreement with El-Alfy *et al.*[35] on polyphenolic extracts tested against streptozotocin-induced diabetes.

During diabetes, increased oxidative stress plays a central role in the development of these complications[36]. However, enzymatic endogenous antioxidants such as superoxide dismutase (SOD), catalase (CAT) and Glutathione (GSH) are responsible for detoxifying the body of these harmful free radicals. This could slow down hyperglycemia and avoid the development of complications due to the onset of diabetes[37]. Evaluation of lipid peroxidation showed a significantly elevated level of cytosolic MDA in the liver in diabetic rats and those not protected by MESO ($p<0.001$). This hyper peroxidation is explained by the fact that streptozotocin is responsible for the formation of free radicals responsible for the oxidation of certain molecules, thus leading to the degeneration of β cells[38]. However, the prior administration of 300 mg kg^{-1} of MESO reduced hepatic MDA levels. These results suggest that polyphenols of *S. officinalis* as well as gallic acid are cellular chemoprotective compounds playing an important role against the deleterious effect of free radicals, which explains the maintenance of the level of MDA at its normal cytosolic level[39].

Current work also shows that pretreatment of animals with MESO and gallic acid significantly increased the concentration of GSH in the liver in diabetic rats. Phenolic compounds are known for their scavenger effect or free radical scavenger, so they can directly participate in reducing the use of GSH[32].

Insufficient antioxidant defenses in the control of ROS can also be caused by decreased activity of SOD and CAT causing significant tissue damage[40]. Several studies have shown that the enzymatic activity of SOD and CAT decreases during diabetes mellitus. These results are consistent with the present study where there was a very significant decrease in SOD and CAT activity. Kakkar *et al.*[41] suggested that hypoinsulinemia produced in diabetes mellitus increases the activity of the fatty acyl-CoA oxidase enzyme, which increases the production of H_2O_2 whose role is to inhibit the activity of SOD. Inactivation of SOD could indirectly play an important role in the activity of CAT, an enzyme involved in the detoxification of hydrogen peroxide[42]. Pretreatment of rats with MESO caused a very highly significant increase in SOD and CAT. Mamdouh and his collaborators showed evidence of the chemoprotective and antiradical effect of polyphenols on antioxidant enzymes in streptozotocin-diabetic rats[43].

It appears from this work that *Salvia officinalis* is a very interesting product rich in therapeutic possibilities. However, this work remains preliminary and not very indicative of the real mechanism by which polyphenols act. Consequently, the purification and identification of these active compounds with antidiabetic and antioxidant activity remains strongly recommended in order to explore in a more specific manner the different actions revealed.

CONCLUSION

In the present work, the preventive activity of the methanolic extract of *Salvia officinalis* against diabetes and its antioxidant activity in diabetic rats was evaluated. The results obtained from this study reveal that these phenolic substances have chemoprotective power against the prooxidant and diabetogenic effect of streptozotocin, which is manifested by maintaining the redox balance of cells. The results obtained showed the beneficial effect of the phenolic compound of *Salvia officinalis* not only against diabetes and associated metabolic disturbances, but also its remarkable effect against the resulting oxidative stress with an emerging probability regarding the prevention of diabetic complications.

SIGNIFICANCE STATEMENT

The regular administration of modern medications causes many side effects. Recently, diabetologists have come to the evidence of a therapeutic supplement consisting of plant extracts in order to optimize the treatment of diabetes. Several types of research are directed towards medicinal plants, considered as an enormous source of multiple phytotherapeutic substances, endowed with both anti diabetic and antioxidant activity. In this sense, the present study aims to evaluate the effect of the methanolic extract of *Salvia officinalis* L. against oxidative stress in streptozotocin-diabetic rats. The results obtained showed the beneficial effect of the phenolic compound of *Salvia officinalis* not only against diabetes and associated metabolic disturbances but also its remarkable effect against the resulting oxidative stress with an emerging probability regarding the prevention of diabetic complications.

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