Effects of Salvia officinalis L. (sag) leaves Extracts in Normal and Alloxan-Induced Diabetes in White Rats

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ABSTRACT: This study was conducted at the laboratories of the faculty of Science / university of Kufa from October 2012 to April 2013. The study was undertaken to investigate the effectn of aqueous and ethanolic extracts of Sage (Salvia officinalis L) leaves at concentration (100) mg/kg in dosage on albino rats for 14 days, on blood glucose , serum cholesterol and triglycerides (TG) level in induced-diabetic rats by alloxan (150) mg/kg compared with the reference drug Glibenclamide, Also, an evaluation of the active commercially available Sage Oil were analyzed by TLC . Results showed significant reduction (P< 0.05) of fasting blood glucose level in alloxan-induced diabetic rats treatment with plant extracts and glibenclamide drug as compared with infected control group. And the sage leaves extracts gave a good results, even better than glibenclamid drug for lowering blood sugar. The results also, showed a slight increase in fasting blood glucose level in normal rats when treatment with plant extracts as compared with healthy control group and showed a significant increase (P< 0.05) in the level of cholesterol compared with the healthy control group , also shown significantly decreased (P< 0.05) in the level of TG when treatment of diabetes rats with alcoholic and aqueous extracts of the plant leaves compared with the healthy control group . Rf values of spots and UV spectra (with and without adding the specific agents) and compared with the literature data it was determined that the isolated compounds were Anetole,Thuijone,Camphor,α-Humulene,α-Terpinol,Gernaiol and Limonene

Keywords: Salvia officinalis, Diabetes mellitus, Alloxan, Blood sugar, Fasting, Alcoholic, Anetole

INTRODUCTION

Diabetes Mellitus is a metabolic disorder characterized by hyperglycemia due to effects in insulin secretion, action or both. Chronic hyperglycemia in diabetes is associated with long term damages, dysfunction and eventually the failure of organs, especially the eyes, kidneys, nerves and cardiovascular system (Vinik & Vinik, 2003). Currently available therapy for diabetes include insulin and various oral anti-diabetic agents such as sulfonylureas, metformin and α-glucosidase inhibitors. Each of the above oral agents suffers from a number of serious adverse effects (Zhang & Moller, 2000; Moller, 2001). Thus, it appears useful to look for new methods in treatment of diabetes. Medical plants are world widely used and many of they were

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es, for der to effect (Pushparaj *et al*., 2000; Alarcon-Aguilar *et al*., 2002; Hosseinzadeh *et al*., 2002; Kameswararao *et al*., 2003; Singh *et al*., 2007). *Salvia officinalis* L. (common sage, garden sage or Dalmatian sage) is a medicinal and aromatic plant of the *Lamiaceae* (= *Labiatae*) family, native to Mediterranean countries, which today is cultivated all over the world (Lima, 2006). The botanical name of sage is a clear reference to the important curative properties of the plant: the genus name *Salvia* comes from the Latin *salvāre* meaning “to save” or “to heal” and *officinalis* means medicinal (Dweck, 2000; Miura *et al.*, 2002). *S. officinalis* is the specie of the genus *Salvia* with the highest EO production (Giannouli & Kintzios, 2000), additionally, many other active compounds that gives it it's medicinal and aromatic properties and makes it a rich source of bioactive compounds (Giannouli & Kintzios, 2000; Dweck, 2000; Barnes *et al*., 2002; Lima, 2006).*Salvia* genus is a rich source of biologically active water soluble components, namely phenolic acids and flavonoids,Caffeic acid , rosmarinic acidand 1,8-cineole, *cis*-thujone, *trans*-thujone, camphor and borneol as a major volatile components (Lima et al., 2005 , Giannouli & Kintzios, 2000) . Common sage, since ancient times, has been an ingredient in perfumes, a flavoring in a variety of food preparations, and a medicinal plant used in folk medicine for the treatment of a variety of ailments (Malamas & Marselo, 1992), where many studies mentioned that sage have many of biological activities, such as antioxidant, antibacterial, hyperglycemic and anti-inflammatory activities (Cherevatyî *et al*., 1980; Baricevic *et al*., 2001; Alarcon-Aguilar *et al*., 2002; Lima, 2006). Also other studies, conducted on Sage extracts and their EO, have shown it's hypotensive properties, anti-spasmodic effect and central nervous system-depressant activities (Newall *et al*., 1996). Addition to therapeutic effects for metabolic and endocrine diseases (Istudor, 2001). *S. officinalis* L. is among the plants that are claimed to be beneficial to

diabetic patients, and previous studies have suggested that some of its extracts have hypoglycemic effects in normal and diabetic animals (Alarcon-Aguilar *et al*., 2002; Eidi *et al*., 2005; Lima *et al*., 2006; Eidi & Eidi, 2009).Within a short period of time thin layer chromatography has become a most important technique for the identification, characterization and determination of chemical compounds as well as complex mixtures. In the authors' laboratory the technique has been used with considerable success for the analysis of essential oils and their constituents. The purpose of the current study was to examine the hypoglycemic effects of aqueous and ethanol extracts of *Salvia officinalis* leaves in normal and alloxan-induced diabetic rats.

MATERILS AND METHODS

Collection and Classification of the plant: Sage was obtained from Mashhad, Iran and the plant samples were identified in botany laboratory, department of biology, Sciences faculty / university of Kufa. The leaves of plant were collected and dried, and then the dried plant samples were ground well into a fine powder and stored in darkish bags for later use. The treatment was conducted at laboratory conditions at the Plant research laboratory / Sciences faculty / university of Kufa. Preparation of aqueous and alcoholic extract : The alcoholic extraction process was conducted according to Hajzadeh and coworkers (2011) , (30g) of dried plant powder was packed in a filter paper type Watman (No.1) and extracted in a soxhlet apparatus using (450 ml) ethanol (90%) at (60 C°) for (13h). After extraction, the extract was filtered and concentrated by rotary evaporator, than dried by oven at (45 C°). The dry material was collected in closed bag and maintained at (4 C°) until use. For preparation of aqueous extract, (25g) of the dried plant powder were suspended in (500 ml) distilled water by rotation magnetic stirrer for an hour and a half, then it left for a period of (24 h). The mixture was filtered by filter paper type Watman (No.1) and centrifuged for (10 min) at (3000 r/m) to remove particulate substances. Then the extract was dried by oven in (45 C°), collected dry material and kept in (4 C°) until use (Harborn, 1984).

Thin layer chromatography analysis and reagents

Vanillin-sulfuric spray reagent was prepared by dissolving 1.5 gm vanillin in 2% ethanolic sulfuric solution(Ciesla *et al* 2001). Thin layer chromatography has been applied to the analysis of essential oils and their constituents from *in vitro* and *in vivo* of medicinal plant (Ciesla *et al* 2001) . R*f*-data are given for terpene compounds. Vanillin sulfuric acid (5% w./v.) is used as spray reagent and sensitivity limits are reported for the compounds examined. . The plates silica gel TLC were then air-dried for twenty minutes and activated by heating in an oven at 100C for 15 minutes ,mobile phase solvent system composed of toluene ;acetate Ethyle solvent system in (7:93) v/v was used (Eukasz M. , 2010). Then placed into a specially designed chamber. The plates were developed to the distance of 90 mm. Then the plates were dried at room temperature for 15 min, prior to derivatization. Heating for 5 min at 105C the chromatoplate after spraying was found to bring about further characteristic color changes and increased moreover the sensitivity of the reagent, making it suitable for the detection of trace constituents . Plate images by the camera visualized under visible and UV light at 254 and 366 nm (Stafford *et al* ,2005) .Tentative identification of spots was achieved by comparison of values with those of authentic reference standards.

Animal Models: Albino rats were used in the experiment, Their weight ranged between (150-250 g), of either sex roughly the same age (4-6) months. These animals were subjected to identical laboratory conditions throughout the period of the research. The animals were divided into seven groups, each group (6) animals (3) males and (3) females: Group (1) dealing with natural food and water were considered as normal control .

Group (2) injected with alloxan drug and left without treatment, given distilled water only for 14 days and considered as diabetic control.

Group (3) normal rats dosage alcoholic extract of the Sage leaves (100) mg/kg of body weight as single dose per day for 14 days.Group (4) normal rats dosage aqueous extract of the Sage leaves (100) mg/kg of body weight as single dose per day for 14 days (Eidi *et al*., 2005; Upendra *et al*., 2011; Ahmadi & Elahe, 2012). Group (5) diabetic rats dosage alcoholic extract of the Sage leaves (100) mg/kg of body weight as single dose per day for 14 days. Group (6) diabetic rats dosage aqueous extract of the Sage leaves (100) mg/kg of body weight as single dose per day for 14 days. Group (7) diabetic rats dosage Glibenclamide drug (0.6) mg/kg of body weight as single dose per day for 14 days (Pari & Umamaheswari, 2000; Eliza *et al*., 2009; Erejuwa *et al*., 2011). The dosage process was taken place by using a plastic tube connected with syringe. After the haling of material (Sag leaves extracts and Glibenclamide drug) to the inside of the tube, given to the animals by the inputting of the plastic tube orally and through the esophagus into the stomach to ensure the entry of the material and the animals take it have fully . Diabetes was induced in rats by alloxan, where animals were starved for 18 hours before being injected with alloxan (150 mg/kg) of body weight (Arbeeny & Bergquist, 1991) with an intraperitoneal single dose (Hadcock *et al.*, 1991), alloxan was prepared before injection directly by using colder

Citrate Buffer. Three days later, it was measured blood sugar level in rats treated with alloxan, animals that showed blood glucose level over (200 mg/dl) considered to have diabetes (Teixeira *et al*., 2002). It has been measuring blood sugar at a rate of once every two days throughout the dosage period (14) days on the same way as above through the wound the vein of guilt to sure the animals does not return to the natural state. At the end of the experiment, which lasted 14 days, The animals were fasted for 12 hours, after which take (3-5 ml) blood from heart by heart puncture. The blood Put in test tubes and left for 30 minutes until clotting, then took to the centrifuge (3000 r/m) for 15 minutes to separate serum and save it in special tubes, after that measured the level of blood glucose by a spectrophotometer and using measuring blood glucose kit.

Statistical Analysis: Results were analyzed statistically using a complete randomized design (CRD), and tested significantly with least significant differences (L.S.D) at level (P< 0.05) to indicate the significant of results (Al-Rawy and Kalafallh, 2000).

RESULTS AND DISCUSSION

The presence of sage plant phytochemical compounds was also detected by thin layer chromatography. TLC is a standard technique, which separates the organic compounds from lower molecular weight according to their polarity (Li *et al*,2004 ; Lima**,**2006**)**. the developing solvent was able to separate different chemicals having different retention factor (R*f* value) present in plant extracts .

The results show in the figure (1C) present (7) spots with R*f* value(0.32,0.37,0.72, 0.74, 0.81, 0.98,1.00) in alcoholic sage leaves extracts and shown 4 spots in TLC plat from aqueous sage leaves extracts(0.81, 0.77,0.74 and 0.32) (Figure 1B) , the results were documented and used for the comparison of the obtained profiles with the fingerprint of the authenticated reference material represent of Anetole,Thuijone,Camphor,α-Humulene,α-Terpinol,Gernaiol and Limonene).Shimoni et al,2003:Ovrar et al,2010with alcoholic extracts and (Limonine,Camphor,Thuijone and and Anethole)with aqueous extracts(Kart-Georg et al,2003, Kosales et al,2005: Citoan et al,2010: ovrar et al,2010.). It is expected that more active compounds can be detected by TLC bioautography, if different solvent systems, from the TLC profiles of essential oil separated ,it appeared that were consisted of more than one constituents. In contrast in the preparation , the result farther suggested that the sage leaves do not seen contain any alkaloids compounds in free form by using Acetone , Water , NH_4OH (90,7,3)v/v/v (Al-Rubaei,,1999) Figure 1 (E). In almost all the investigated samples the presence of band corresponding to identical compounds was indicated with an arrow *S.officinals* plant.

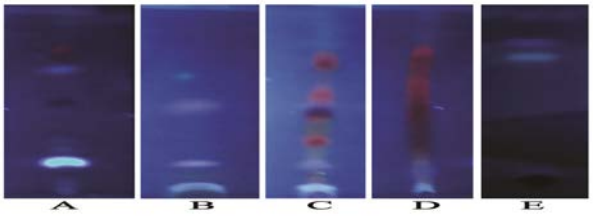

Figure 1: Basic properties of chromatographically isolated compounds from *S. officinals leaves* alcoholic and aqueous extracts
C. R*f* value (0.32,0.37,0.72, 0.74, 0.81, 0.98,1.00)
B. R*f* value (0.81, 0.77,0.74 , 0.32)
E.R*f* value (no spots)

Estimation of the serum blood sugar level in normal and diabetic rats:

Results (Table 1) was indicated that the induced of diabetes in experimental animals led to a significant increase (P< 0.05) in the level of blood sugar (410.25 ± 6.38 mg/dl) compared with the healthy control group (89.25 ± 1.89 mg/dl), Results have shown presence significantly decreased (P< 0.05) in the level of blood sugar when treatment of diabetes rats with alcoholic and aqueous extracts leaves plant as it was reaching (209 ± 5.87 mg/dl) and (209.5 ± 3.86 mg/dl), respectively, as well as they treatment with Glibenclamide drug (255 ± 9.33 mg/dl) compared with the infected control group. But alcoholic and aqueous extracts gives significantly decrease (P< 0.05) in the blood sugar level more than it by using Glibenclamide drug.

Also, the results, showed occurring a significant increase (P< 0.05) in the level of blood sugar when treatment of healthy animals with alcoholic and aqueous extracts (95.75 ± 2.78 mg/dl) and (92 ± 2.48 mg/dl), respectively, compared with the healthy control group.

These results agreed with other studies conducted to investigate the effect of sage on hyperglycemia by other researchers using a different extracts and experimental methodology (Alarcon-Aguilar *et al*., 2002; Eidi *et al*., 2005; Eidi & Eidi, 2009).

Estimation of the serum blood sugar level in normal and diabetic rats:

As The results (Table 2) was indicated to the presence of a significantly increase (P< 0.05) in the total cholesterol level in diabetic control animals (112.2 ± 2.21 mg/dl) compared with the healthy control group (85.125 ± 1.89 mg/dl), these results agreed with what the many researchers have been reached (Bopanna *et al*., 1997; Al-A'miri, 2003), This cholesterol rise may be to increase the cholesterol absorption by intestine due to increase activity of Acyl-Co-A: Cholesterol Acyl transferase that stimulated in the insulin absence, and the absence of insulin leads to decrease of ApoE mRNA level thereby increasing the total cholesterol level (Maechler *et al*., 1993). In another explanation, the high level of cholesterol as a result of diabetes gets as a result of oxidation and the glycation process that occur on (LDL-C) or their receptors, where the large quantities of the cholesterol carried on (LDL-C) (Durlach *et al*., 1996). The treatment of diabetes rats with alcoholic and aqueous extracts of sage leaves, it has led to obtain significant decrease (P< 0.05) in the total cholesterol level (88.125 ± 2.29 mg/dl) and (87.375 ± 1.14 mg/dl), respectively, as well as they treatment with Glibenclamide drug (94.15 ± 2.78 mg/dl) compared with the infected control group, but the decrease in the total cholesterol level as a result of treatment with a Glibenclamide drug less than it when treatment with alcohol and aqueous extracts of plant leaves. The results showed, also, a significant decrease (P< 0.05) in total cholesterol level of healthy animals groups that treatment with alcoholic and aqueous sage leaves extracts as it was (81.6 ± 1.06 mg/dl) and (81 ± 1.06 mg/dl), respectively, when compared with the healthy control group. This agree with the results of Alayan (2006), Khattab and his group (2012) and Behradmanesh and his group (2013), which have shown the activity of sage plant in reducing the serum cholesterol level.The reason of this, perhaps due to the containment of sage leaves on high percentage of active components lectin and saponin, that responsible for hypolipidemic effects (Alayan, 2006). Lectin is proved to have a significant effect in lowering both serum and hepatic cholesterol (Okazaki *et al*., 2005), Sauvaire and his group (1996) explained that the saponin reducing effect of the serum cholesterol level, may be due to the decomposition of saponin to sapognin in the gastrointestinal tract, which activates the secretion of bile acids by the liver. It was noted that saponin have formed with cholesterol insoluble complexes in the cavity of gastrointestinal tract, and these complexes inhibit the cholesterol absorption from the intestine, leading to excrete it with waste and thus lower its level in the blood. Also, the saponin have ability to sticking with bile acids and neutral fat in the intestine and inhibited its absorption, and then reduced its level in the blood and stimulate the liver to convert cholesterol into bile acids (Spiller, 1996). This influence effective of *S. officinalis* on the level of total cholesterol may be due to its contain sterols (Newall *et al*., 1996; Capasso *et al*., 2003), which showed many of studies to be effective in reducing the level of total cholesterol in the blood (Moreau *et al*., 2002). As for the level of triglycerides, the results of table (2) showed there is a significant increase (P< 0.05) in triglycerides level of the diabetic control group as it was (104 ± 2.20 mg/dl) compared with the healthy control group (63.575 ± 2.78 mg/dl), and this agreed with study of Pari & Latha (2002) and Al-A'merri (2003). This rise returns to the metabolic disorders that associated of diabetes mellitus, where the body depends on the analysis of fats in adipose tissue to fill its needs of energy because inability to use of the high glucose that found in the blood, thus the result be the high level of serum TG (Howard, 1999). And the lack of insulin leads to inhibition the activity of the enzyme Lipoprotein Lipase (LPL), which causes the reduction of the process of triglycerides removing in chilomicrone and VLDL result to be converted into fatty acids and glycerol, and therefore

TABLE 1

TABLE 1: EFFECT OF SAGE LEAVES EXTRACTS ON BLOOD SUGAR LEVEL IN NORMAL AND DIABETIC RATS.

Groups	Blood sugar level mean ± S.E.M.
Normal control	89.25 ± 1.89
Normal + Alcoholic extract	95.75 ± 2.78
Normal + Aqueous extract	92 ± 2.48
Diabetic control	410.25 ± 6.38
Diabetic + Alcoholic extract	209 ± 5.87
Diabetic + Aqueous extract	209.5 ± 3.86
Diabetic + Drug	255 ± 9.33
LSD (P< 0.05)	2.63

rising its level in the blood (Bishop, 2000). When the diabetes rats was treatment with alcoholic and aqueous extracts of sage leaves has led to obtain significant decrease ($P< 0.05$) in the triglycerides level where its level reached (65.7 ± 1.67 mg/dl) and (63.25 ± 1.76 mg/dl), respectively, as gets this decrease when the diabetes rats had treatment with drug (70 ± 1.83 mg/dl) compared with the infected control group, but the aqueous and alcoholic extracts gave a significant decrease ($P< 0.05$) in the triglycerides level larger than the level of decrease by using the glibenclamide drug. The triglycerides level of healthy animals groups that treated with alcoholic and aqueous extracts of plant leaves has decrease significantly ($P< 0.05$), as was (59.5 ± 1.43 mg/dl) and (60.375 ± 1.52 mg/dl), respectively, compared with the healthy control group.

This is agrees with several studies that reported the impact effective of the plant in reducing the triglycerides level (Carla *et al*., 2009; Kianbakht *et al*., 2011; Khattab *et al*., 2012), but its disagree with what Alayan (2006) was reached, which reported lack the significant effect of sage aqueous extract on TG level in the blood. This lowering of triglycerides levels in treatment animals with *S. officinalis* extracts, due to the anti-oxidative role of the plant, It became clear his role effectively in preventing lipid peroxidation therefore prevent the lyses of lipid (Cuppett & Hall, 1998; Miura *et al*., 2002; Jaswir *et al*., 2005), as diabetes is one of the factors that cause oxidative stress (West, 2000).

Also, Carla and his group (2009) and Kianbakht and his group (2011) reported that this effect may be due to the ability of *S. officinalis* to suppress the cholesterol biosynthesis, as a result of it contains active compounds have effected on the cholesterol metabolism by reducing its absorption or synthesized such as Thujone, which decrees the level of cholesterol and triglycerides.

The phytosterols in sage plant also have an impact affected on the triglycerides level, where many of the studies confirmed the effective of these compounds in reducing the level of serum triglycerides (Plat & Mensink, 2009). In addition, the improvement that made in the level of blood glucose and increase insulin sensitivity as a result of treatment with sage leaves extracts and glibenclamide drug, lead to correct metabolic pathways and reduce the lyses of lipid in the tissues therefore decreases its level in the blood, this is referred to it by Swenson (1991). The results (Table 2) indicated also, that the aqueous extract of Sage leaves gave better effect in reducing the level of blood lipids than alcoholic extract and drug.

CONCLUSIONS

Our results showed significant reduction ($P< 0.05$) of fasting blood glucose level in alloxan-induced diabetic rats treatment with plant extracts and glibenclamide drug as compared with infected control group. And the sage leaves extracts gave a good result, even better than glibenclamid drug for lowering blood sugar. The results also, showed a slight increase in fasting blood glucose level in normal rats when treatment with plant extracts as compared with healthy control group and showed a significant increase ($P< 0.05$) in the level of cholesterol compared with the healthy control group , also shown significantly decreased ($P< 0.05$) in the level of TG when treatment of diabetes rats with alcoholic and aqueous extracts of the plant leaves compared with the healthy control group . R*f* values of spots and UV spectra (with and without adding the specific agents) and compared with the literature data it was determined that the isolated compounds were Anetole,Thuijone,Camphor,α-Humulene,α-Terpinol,Gernaiol and Limonene. Our results also indicated that improvements made in the level of blood glucose and increase insulin sensitivity as a result of treatment with sage leaves extracts and glibenclamide drug, lead to correct metabolic pathways and reduce the lyses of lipid in the tissues therefore decreases its level in the blood, also, the aqueous extract of Sage leaves gave better effect in reducing the level of blood lipids than alcoholic extract and drug.

TABLE 2
TABLE 2 : EFFECT OF SAGE LEAVES EXTRACTS ON CHOLESTEROL AND TG LEVELS IN HEALTHY AND DIABETIC RATS

Groups	***Cholesterol and TG levels*** *mean ± S.E.M.*		
		Cholesterol	***TG***
Normal control	*85.125 ± 1.89*	*2.78 ± 63.575*	
Normal + Alcoholic extract	*81.6 ± 1.06*	*1.43 ± 59.5*	
Normal + Aqueous extract	*1.52 ± 60.375*	*1.52 ± 60.375*	
Diabetic control	*112.2 ± 2.21*	*2.20 ± 104*	
Diabetic + Alcoholic extract	*88.125 ± 2.29*	*1.67 ± 65.7*	
Diabetic + Aqueous extract	*87.375 ± 1.14*	*1.76 ± 63.25*	
Diabetic + Drug	*2.78 ± 94.15*	*1.83 ± 70*	
LSD(P< 0.05)	*3.26*	*2.63*	

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