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PREPARATION OF SALVIA OFFICINALIS L. EXTRACT AND STUDY OF ITS EFFECT ON SOME PHYSIOLOGICAL VARIABLES IN ALBINO RATS

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ABSTRACT

*Salvia officinalis* is one of the most valued herbs because of its high amount of essential oil and its many components. It has many health-related uses such as improving cognition, as well as reducing the amount of nursing mother's milk during weaning, also recommended for the gargling of the infectious throat, and to treat cardiovascular diseases and nervous disturbance, depression, cerebral ischemia and acts as an antiseptic for wounds. This study aim was to prepare the plant extract of *Salvia officinalis* L. from the Iraq local areas, determine its chemical properties and study its effect on some physiological and immunological variables in white rats. The plant leave Extract was Prepared and its effects were assessed on several physiological parameters using 20 male rats. The rats were divided into four groups. The control group (G1) received standard food and water; the negative control group (G2) received fat rich diet; G3 rats were given *Salvia officinalis* with a concentration of 100 mg\kg\day. G4: rats were given *Salvia officinalis* 100mg/kg body weight and received fat rich diet. Roche/Hitachi, cobas c 501/502 were used to evaluate the levels of aminotransferase Enzymes, Aspartate transaminase (AST), Alanine aminotransferase (ALT), alkaline phosphatase enzyme (ALP), serum creatinine, serum urea, total protein, albumin, cholesterol, triglyceride, and high-density lipoprotein (HDL). While Globulin concentration, very Low-Density Lipoprotein (VLDL), and Low-Density Lipoprotein (LDL) were calculated according to some referred equations. The phytochemical analysis showed that the compounds identified in the sample were found to be alkaloids, phenols, tannins, coumarins glycosides, flavonoids, quinines, Carbohydrates and steroids. The results showed a significant increase (P <0.01) in the level of liver enzymes ALT, AST and ALP, in the G2 treated with the high fat diet compared with a control group and showed a significant increase (P <0.01) in the level of urea and creatinine in animal serum treated with high fat food compared to the control group. Animals administrated with *Salvia officinalis* (G3 and 4) were significantly increase (P <0.01) the levels of the levels of Total Protein, Albumin, and Globulin in comparison with rats in the G2 group. Administration with *Salvia officinalis* in G4 group reduced (P <0.01) the Triglycerides, (VLDL-C), and (LDL-C) and markedly enhanced HDL levels (P <0.01) in G3 group compared with G4 group.

Keywords: *Salvia Officinalis*, Extraction, Rat, Cholesterol, Triglycerides, HDL-c.

INTRODUCTION

*Salvia officinalis* (Sage) is a multipurpose culinary herb belonging to the families of Lamiaceae/Labiatae. It is also known as Gardensage, Common Sauge or Culinary Sauge. It is a fragrant, permanent, woody subshrub that comes from the northern Mediterranean region and is extensively spread throughout the slopes and beaches.
of Southern Europe. It is cultivated in Europe and the United States, Spain and Italy in particular [1] [2]. It has been utilized as a culinary herb in many nations and is currently grown worldwide for dried leaves which are used as a raw material for the pharmaceutical, perfume and food industries. [3].

It is one of the most valued herbs because of its high amount of essential oil and its many components [4]. It has many health-related uses such as improving cognition, as well as reducing the amount of nursing mother's milk during weaning, also recommended for the gargling of the infectious throat, and to treat cardiovascular diseases and nervous disturbance, depression, and cerebral ischemia. It acts as an antiseptic for wounds [5][6][7].

The species is a well-recognized plant in the food industry, pharmaceutical industry and cosmetics industry. It's a well-known herb. The areal portions (Salvia folium) are included as a natural source for food flavour, in various European Pharmacopeias [8]. And was serving as an antidiabetic [9][5], antioxidant [10], anti-inflammatory [11], antimicrobial [12], antiviral [13], gastroprotective and antimutagen [14] agent. It has also been shown to be beneficial in the treatment of cardiovascular and cancer disorders.[15][16] as well as in the treatment of depression and neurological disorders [17][18]

S. Officinalis has been employed in both traditional medicine and clinical studies to treat menopausal symptoms. Hot flashes, sleeplessness, night-time sweating, dizziness, migraines, and palpitations are some of the symptoms. [19][20]

S. Officinalis leaf preparations have long been used as a diabetic treatment. Swanston-Flatt et al. examined the possible anti-diabetic effects of S. Officinalis extract against type I and type II diabetes [9]. According to their findings, ethanol extracts of S. Officinalis considerably lower blood glucose in healthy rats and hyperglycemia in type I diabetic rats [21]. Furthermore, Eidi et al discovered that the methanolic extracts of S. Officinalis reduces blood glucose in type I diabetes while having no effect on insulin production by pancreas [22]. Lima et al. discovered that a tea preparation of S. Officinalis decreases glucose production by the liver while increased insulin action in type II diabetes, comparable to metformin, which is commonly used in the therapeutic treatment of diabetes [23]. Patenkovi et al. revealed the possible antimutagenic activity of S. Officinalis tea, which may
operate via suppressing metabolism via antioxidative action [24].

Fresh S. Officinalis extracts used once daily exhibited good therapeutic value in terms of safety, effectiveness, and tolerance in the treatment of menopausal symptoms. S. Officinalis is also renowned for its ability to help with memory problems, depression, and cerebral ischemia [25]. S. Officinalis as well as used to treat Alzheimer’s disease, with essential oil acting as an acetylcholinesterase (that might play a major role in the memory loss) inhibitors, and increasing acetylcholine, a neurotransmitter component involved in signal transmission among synapses [23]. A number of studies have shown indicated that S. Officinalis enhances memory and cognition [17].

S. Officinalis is safe to use at prescribed doses, and there have been no complaints of harmful side effects [26]. However, recommended dosages should thus never be overrun and S. Officinalis formulations should never be taken for longer durations. The negative impact could appear due to the high thujone concentration [11]. Furthermore, the overuse of its products caused many disorders for the nervous as well as dementia and seizures [27]. The aim of current study to prepare the plant extract of Salvia officinalis L. from the Iraq local areas, determine its chemical properties and study its effect on some physiological and immunological variables in white rats.

MATERIALS AND METHODS

Preparation of Extract

The leaves of Salvia officinalis were obtained from Mosul market’s/Iraq. The dried leaves Salvia officinalis was converted in to powder with the help of grinder for 30s. The dry leaves powder sample (15gm) was extracted using 80% methanol solvent (250 ml) for 24hrs using Soxhlet extractor until complete extraction. After extraction, the methanol extract was evaporated using a rotary evaporator under pressure for 30 min the resulting is solid crude extract. Then dry crude extract weighted (3.41 g) was transferred into test-tube for antioxidant activity, and phytochemical screening. [28]

Phytochemical Screening

Phytochemical screenings of Salvia officinalis extract were assessed by standard methods. These include detection the founding of alkaloids, terpenoids, phenols, tannins, carbohydrates, saponins, glycosides, flavonoids, quinines, and steroids. [29][30].
Assessment of *Salvia officinalis* effects

Male albino rats (20 rats) were used to assess the effects of *Salvia officinalis* in vivo on the physiological parameters. The lab animals were obtained from the Central Animal House Facility of Zakho University. Their ages ranged from 7 to 9 weeks and weight: (180 - 200 g). The rats were kept in the filter-protected and air-conditioned room with constant temperature (21–25 c°), the humidity of 50–60%, and photoperiod of 12 h. After one week of acclimatization, were randomly divided into six groups each group contains 3 animals.

The first group (G1: the control group) received standard food and water. The second group was (G2) the negative control group received the animal’s food and fat (30%) [31] for 2 months. G3: (*Salvia officinalis* group) rats were given *Salvia officinalis* with a concentration of 100 mg/kg/day [31] for 2 months. G4: (Salvia officinalis + fat rich diet) rats were given *Salvia officinalis* 100mg/kg body weight and received fat (30%) rich diet for 2 months.

**Animal Anesthesia and Dissection**

At the end of the study, the study rats were anesthetized using chloroform and then compassionately slaughtered. Blood was collected and centrifuged to obtain the serum in order to evaluate the other parameters of the study.

**THE STUDY PARAMETERS**

**Measurement of some study parameters.**

Concentrations of Aminotransferase Enzymes AST, ALT, alkaline phosphatase enzyme ALP, serum creatinine, serum urea, total protein, albumin, cholesterol, triglyceride, and high-density lipoprotein (HDL) were measured using (Roche/Hitachi, cobas c 501/502).

**Calculation of some study parameters:**

Globulin concentration, VLDL-C, and LDL-C, concentration was calculated according to the following equations.

Globulin= Total Protein con. -Albumin con. [32],[33]

\[ \text{VLDL-C} = \frac{\text{Triglycerides concentration}}{5} \] [34]

\[ \text{LDL-C}= \text{cholesterol} - (\text{HDL} + \text{VLDL-C}). \] [34],[35].

**Statistical analysis:**

SPSS statistical program was used to analyze the results of the experiments. The ANOVA Duncan test was used to compare
the studied groups and the control group. The $p < 0.01$ were used for mean comparisons.

**RESULTS**

**Characterization of bioactive compounds**

The extract was subjected to phytochemical analysis. This resulted in the determination of bioactive compounds that were present in the methanol sample of plant extract. The compounds identified in the sample were found to be alkaloids, phenols, tannins, coumarins glycosides, flavonoids, quinines, Carbohydrates and steroids. The results are shown in table 1.

**Table 1.** Phytochemical analysis of *Salvia officinalis* extract.

<table>
<thead>
<tr>
<th>Phytoconstituents</th>
<th>methanol extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>+ve</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+ve</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>-ve</td>
</tr>
<tr>
<td>Tannin</td>
<td>++ve</td>
</tr>
<tr>
<td>Steroids</td>
<td>+ve</td>
</tr>
<tr>
<td>Phenols</td>
<td>+ve</td>
</tr>
<tr>
<td>Coumarine glycoside</td>
<td>+ve</td>
</tr>
<tr>
<td>Quinine</td>
<td>+ve</td>
</tr>
<tr>
<td>Protein</td>
<td>-ve</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>+ve</td>
</tr>
<tr>
<td>Saponine</td>
<td>-ve</td>
</tr>
</tbody>
</table>
Liver Enzymes

The results of the statistical analysis showed a significant increase (P <0.01) in the level of liver enzymes ALP, ALT, AST in the G2 group treated with the high fat diet compared with a control group.

Urea and Creatinine level

The results showed a significant increase (P <0.01) in the level of urea and creatinine in animal serum treated with high fat food compared to the control group.

Table 2. Showing the effect of the *Salvia officinalis* extract on some liver enzymes and urea and creatinine in white male rats of the study

<table>
<thead>
<tr>
<th>Groups</th>
<th>Cr (mg/dl)</th>
<th>Urea (mg/dl)</th>
<th>ALP (U/L)</th>
<th>ALT (U/L)</th>
<th>AST (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>0.543 ± 0.0277d</td>
<td>18.532 ± 0.255d</td>
<td>82.85 ± 0.203b</td>
<td>11.661 ± 0.126d</td>
<td>16.877 ± 0.175c</td>
</tr>
<tr>
<td>G2</td>
<td>0.962 ± 0.01a</td>
<td>45.194 ± 0.22a</td>
<td>91.11 ± 0.014a</td>
<td>26.43 ± 0.024a</td>
<td>28.563 ± 0.027a</td>
</tr>
<tr>
<td>G3</td>
<td>0.602 ± 0.06c</td>
<td>34.919 ± 0.247b</td>
<td>73.55 ± 0.017c</td>
<td>16.543 ± 0.06c</td>
<td>17.518 ± 0.131c</td>
</tr>
<tr>
<td>G4</td>
<td>0.723 ± 0.05b</td>
<td>23.921 ± 0.154c</td>
<td>83.822 ± 0.015b</td>
<td>19.864 ± 0.09b</td>
<td>21.869 ± 0.07b</td>
</tr>
</tbody>
</table>

Note: Different letter (a,b,c..) within each column indicate significant differences (p ≤ 0.01), Data are presented as the means ± standard deviation, (G1) normal control group, (G2) fat diet group, (G3) *Salvia officinalis* group, (G4) *Salvia officinalis* + fat diet group, There were 5 rats in each group of experiment.
Effect of *Salvia officinalis* on Total Protein, Albumin, and Globulin levels.

Table 3, shows that administration of fat diet in G2 significantly reduced the levels of Total Protein, Albumin, and Globulin in comparison with the normal rats G1 group (p < 0.01), while animals administrated with *Salvia officinalis* (G3 and 4) were significantly increase the levels of the mentioned parameters in comparison with rats in the G2 group.

Table 3. Effects effect of the *Salvia officinalis* extract on Serum Total Protein, Albumin, and Globulin levels.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Total Protein (g/dl)</th>
<th>Albumin (g/dl)</th>
<th>Globulin (g/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>7.120 ±0.34a</td>
<td>5.370 ±0.01a</td>
<td>1.750 ±0.16b</td>
</tr>
<tr>
<td>G2</td>
<td>4.450 ±0.39c</td>
<td>3.320 ±0.01d</td>
<td>1.130 ±0.01c</td>
</tr>
<tr>
<td>G3</td>
<td>6.980 ±0.19a</td>
<td>4.820 ±0.01b</td>
<td>2.160 ±0.01a</td>
</tr>
<tr>
<td>G4</td>
<td>5.983 ±0.01b</td>
<td>4.110 ±0.01c</td>
<td>1.873 ±0.015b</td>
</tr>
</tbody>
</table>

Note: Different letter (a,b,c..) within each column indicate significant differences (p ≤ 0.01). Data are presented as means ± standard deviation.

( G1) normal control group, (G2) fat diet group, (G3) *Salvia officinalis* group, ( G4) *Salvia officinalis* + fat diet group, There were 5 rats in each group of experiment.
Effect of *Salvia officinalis* on lipid profile and Phospholipids levels.

Table 4 shown that the effects of *Salvia officinalis* on lipid profile and Phospholipids in serum (p <0.01) the results indicated that administration of fat diet in G2 group has a negative effect on High density lipoprotein cholesterol (HDL), while it elevated the Cholesterol, Triglycerides, very low-density lipoprotein cholesterol concentration (VLDL-C), low density lipoprotein cholesterol concentration (LDL-C) and Phospholipids levels. However, administration with *Salvia officinalis* in G4 group reduced those Triglycerides, (VLDL-C), and (LDL-C) levels. Furthermore, the intervention of *Salvia officinalis* markedly enhanced HDL levels in G3 group compared with G4 group.

**Table 4**: Effect of the *Salvia officinalis* extract on Cholesterol, Triglycerides, HDL, VLDL-C, and LDL-C.

<table>
<thead>
<tr>
<th>Groups</th>
<th>TC (mg/dl)</th>
<th>TG (mg/dl)</th>
<th>HDL (mg/dl)</th>
<th>VLDL-C (mg/100ml)</th>
<th>LDL-C (mg/100ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>46 ±1.65c</td>
<td>48 ±1.05c</td>
<td>28 ±2.12c</td>
<td>9.6 ±0.210c</td>
<td>8.4 ±0.200c</td>
</tr>
<tr>
<td>G2</td>
<td>73 ±1.80a</td>
<td>76 ±1.30a</td>
<td>26 ±1.83d</td>
<td>15.2 ±0.26a</td>
<td>31.8 ±0.200a</td>
</tr>
<tr>
<td>G3</td>
<td>53 ±1.02b</td>
<td>41 ±1.03d</td>
<td>39 ±1.92a</td>
<td>9.2 ±0.206c</td>
<td>5.8 ±0.200d</td>
</tr>
<tr>
<td>G4</td>
<td>56 ±1.65b</td>
<td>67 ±1.05b</td>
<td>33 ±1.55b</td>
<td>13.4 ±0.210b</td>
<td>9.6 ±0.200b</td>
</tr>
</tbody>
</table>

Note: Different letter (a,b,c,..) within each column indicate significant differences (p ≤ 0.01), Data are presented as the means ± standard deviation, (G1) normal control group, (G2) fat diet group, (G3) *Salvia officinalis* group, (G4) *Salvia officinalis* + fat diet group. There were 5 rats in each group of experiment.
DISCUSSION

The current investigation results have shown that the liver enzymes (ALT and AST) and alkaline phosphatase levels have significantly increased. These findings agreed with research [31], which proved this by examining the tissue samples of the liver and found necrosis that might result in the bloodstream release of these enzymes [36]. High-fat diets have a favorable connection with liver illness via steatosis and a rise in saturated fatty acids in the liver, which coincide with acute hepatic tissue damage, boosting the release of liver enzymes into the circulation, as shown by Zhang and his colleagues [37]. This might be explained by the presence of oxidative damage and free radical production in hyperlipidemic mice [38]. A high-fat diet promotes peroxidation in the liver, heart, and kidneys, and the liver is rich in mitochondrial tissue. As a result, it is much more vulnerable to free radical’s damage, which affects the function of the mitochondria and impairs the electron transport chain efficiency, and leads to the apoptosis process [39].

The alterations produced by elevated MDA in the liver cells, which harm mitochondrial activities, cause oxidation process of nucleotide, lipid, and endoplasmic reticulum enzymes, specifically glucose-6-phosphatase and cytochrome p-450. As a consequence, numerous enzymes are released out of the lysosomes, increasing the plasma membrane permeability and, causing damage[40] to hepatic cells and, as a result, the releasing of their enzymes. The current findings, on the other hand, revealed a significant (P < 0.05) reduction in the level of the liver enzymes in the groups that were treated with Salvia officinalis leaf extract compared to the T1 group fed a fat-rich diet [41]. These findings support a previous study that found Salvia officinalis leaf extract to be effective versus hepatocyte damage in mice fed a high-fat diet [42].

The decreased level of liver enzymes in rats treated with leaf extract is attributable to the plant’s antioxidant components and their involvement in safeguarding organs and tissues from damage associated with oxidative stress produced by a high-fat diet [40].

Antioxidants, such as phenolic and flavonoid compounds, protect lipids in cellular membrane from severe oxidation and breakdown, preventing enzymes from being discharged into the serum [43]. That might be attributable to catechol, which
is a contributor to the synthesis process of rosmarinic acid and caffeic acid, which is recognized to demolish free radical damage in the body cells by shattering free radical chains through blocking the initiation and thus preventing the growth and reproductive capacity of the free radical’s chain, and led to preventing the damage to hepatocytes and sustains the enzymes release into the bloodstream [44].

S. officinalis also provides zinc and selenium and prevents oxidative damage to the liver and kidneys produced by excess free radicals by boosting the potential of antioxidants like SOD, GSH, and GR [45]. It was also discovered that vitamin E and selenium were added to the dietary of the lab animals contributed to reducing cirrhosis through both the blocking and reduction of the hepatic satellite cells (HSC) which has an important role in the cirrhosis formation [46]. The zinc in the S.offisinalis is also playing an essential role in the metallothionine (MT) production, (a protein-rich in the cysteine amino acid), through a linkage between the thiol group of the cysteine terminals and the heavy metals in and therefore increase the antioxidant characteristics [47].

These results are agreed with Ahangarpour et al., that the rise in concentrations of creatinine and urea is due to higher total lipid oxidation [48] and, subsequently, lower concentrations of CAT and SOD enzymes, increasing the generation of oxidative agents resulting in oxidative stress [38].

Yao and his group Yao et al., noted in their in their investigation of the obesity effects on kidney function in rats model on the administration of high-fat meals Yao and his colleagues have hypothesized that kidney disorders induce renal macrophages inflammation and hypertrophy[49].

In another study, there was a considerable rise in blood cholesterol, triglycerides, LDL, and VLDL levels in high-fat diets compared to the control group and the findings of the study showed that the level of HDL-C decreased substantially when compared to the control group [50].

Furukawa et al. found an increase in morbidity in high-fat diets in rats and they proposed that this increases in morbidity can be ascribed to the fact that high-fat diets in rats produce oxidative stress, which can result in significant quantities of reactive oxygen (ROS) species [51].
Munshi et al notes that high-fat diets induced high levels of cholesterol, LDL, triglycerides and VLDL with lower HDL because of an enhanced lipid peroxidation owing to retarded gastric drainage. This was verified in the same study that the build-up of the tiny lipids in the gut, which disrupts the mechanisms of antioxidant defense causing oxidative stress[52]. This might be due to the increase in the quantity of cholesterol in the diet, which would lead to a rise in the efficiency of the cholesterol absorption by the small intestine [53] resulting in an increase of fat accumulation in the liver, which enhances hepatocyte processing [54]. A fat diet can also induce a reductase (HMG-CoA reductase) called 3-methylglutaryl coenzyme A reductase, largely responsible for cholesterol production [55]. The decreased in HDL is likely related to a high fat diet, which decreases the activity of L-CAT (lecithin-cholesterol acyl transferase), a binding enzyme that plays an essential part in the production of HDL, and cholesterol flow from cell membranes toward the HDL [56].

The cause of the Salvia officinalis beneficial effect on lipid profile can be attributed to the contains potent compounds that block triglycerides production, such as single turbines, which are known to be thujone compound, recognized for reducing cholesterol and triglycerides. The drop in lipid concentrations can also be caused by the plant possessing flavonoids which reduce blood lipids as an antioxidant. Flavonoids also serve a direct function in enhancing LDL receptors in the liver and their interaction with protein B. Consequently, removes it from the blood. [43]. Interestingly, the hypolipidemic actions of S officinalis were comparable to hypolipidemic medications in current use [57],[58],[57],[59]; with more tolerable adverse effects profile compared to many chronic drugs in current use

CONCLUSIONS:
A conclusion can be drawn that Salvia officinalis alcohol extracted has a beneficial effect on the lipid profile especially and all the other parameters of the study parameters for all of the extract-treated groups (G3 and G4) compared to the negative control fat diet group (G2)
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