Enzymatic Effective of Alcoholic and Aqueous Extract of *Salvia officinalis* in Mice Poisoned with CCl4

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Abstract

*Salvia officinalis* extracts showed antioxidant, anti-inflammatory and antibacterial activity, and due to the use of medicinal herb as an alternative to chemical drugs to reduce damage of hepatocyte, in this study, enzymatic changes after treated of aqueous and alcoholic extract of *Salvia officinalis* in mice poisoned with carbon tetrachloride were evaluated. 40 adult male mice divided into 8 groups that included 6 experimental and negative and positive control groups, which were exposed to CCl4 at a concentration of 2.3 mg / kg. The active compounds in the alcoholic and aqueous extracts of *S. officinalis* using by HPLC and then mice were given *S. Officinalis* extract orally 100, 200 and 300 mg / kg for six days. The enzymes (GST, ALP, ALT, AST and MDA) were determined in serum. The result of the study showed that enzyme activity had significant decreased in the group treated with *S. officinalis* extract and the concentration 300 mg / kg has the most effectiveness. Also, showed that the alcoholic extract has a higher effect than the aqueous extract that may be because the alcoholic extract contains a greater amount of active compounds. The improving effects of *S. officinalis* can be attributed to the bioactive components as antioxidant properties that inhibit the damaging effects of free radicals, chemical drugs and tissue damage.

Keywords: *Salvia officinalis*, enzymatic effective, alcoholic extract, aqueous extract, CCl4
1. Introduction

*Salvia officinalis* (Sagebrush) is a plant in the family of Labiatae, which is a small perennial herbaceous plant with branches that on average rise about 30 cm above the ground, with the green spot becomes dark red with increasing age. *Salvia officinalis* was originally cultivated in the Middle East and the Mediterranean. Due to the use of this plant as one of the most famous and oldest plants in ancient and modern medicine, today the cultivation of this plant is common throughout the world (1).

One of the therapeutic effects of Sagebrush includes antioxidant effects, that is important compound of the volatile oil (Thujone), and also contain flavonoids and phenolic acids and galls materials (2, 3). In addition to being used as an antiseptic and aromatic agent in the treatment of infections, muscle cramps and mild indigestion (such as heartburn and bloating), excessive sweating, age-related cognitive disorders and inflammation in the throat and skin were used (4, 5).

Comparative effect of aqueous and alcoholic extract of *S. officinalis* as anti-inflammatory showed that alcoholic extract of *S. officinalis* has a higher efficacy than aqueous extract against *Streptococcus mutans*, the alcoholic extract showed the toxicity test results for *S. officinalis* is non-toxic (4, 6).

The liver plays an important role in protecting the body against chemicals. Due to the increase in environmental pollutants following the development of the chemical industry, the production of drugs and pesticides that are considered harmful to human health, liver toxicity base on various mechanisms such as necrosis and fatty liver is common. (7). The increase in the activity of liver enzymes can occur as a result of the increase in synthesis processes in the cell or as a response to the growth processes occurring in the cell (8). Alkaline phosphatase (ALP), AST and ALT are found in various parts of the body and are concentrated in the liver and kidneys and are used to measure or determine the extent of injury to hepatocytes (8). On the other hand, antioxidants have been shown to increase the activity of the enzyme glutathione-S-transfer (GST) which is one of the most important detoxication processes and multifunctional enzyme by removing the toxic metabolites of some carcinogens and mutagens. (8, 9).

As for Malondialdehyde (MDA), final products of the mechanics of oxidation of polyunsaturated and polyunsaturated fatty acid, which has a high toxicity and
effective inhibiting antioxidant enzymes as well as it acts as a tumor initiator and pro-cancer agent (10). MDA leads to the production of free radicals by the process of lipid oxidation, resulting in damage to the unsaturated fatty acids in the cell membrane. That is usually occurs by the process of self-oxidation of acids in the cell (11).

This study aimed to study the effect of the alcoholic and aqueous extract of *S. officinalis* on AST, ALT, GST and MAD in serum of male laboratory mice poisoned with CCl₄ carbon tetrachloride.

2. Materials and Method

2.1. Preparing the alcoholic and aqueous extracts of *S. officinalis*

Alcoholic extract of *S. officinalis* leaves was obtained by 70% ethyl alcohol using Soxhlet extraction machine and dried using a rotary evaporator at 50 °C. Aqueous extract was obtained using distilled water according to the above technique. Wavelengths were determined to detect alcoholic extracts of 278, 356 nm and 305 nm for aqueous extracts by UV visible spectrophotometer. The active compounds in the alcoholic and aqueous extract of *S. officinalis* were measured using the HPLC type Shimadz A6 and the column type ODS CR18 (12). A. Mobile phase: A composed of anionic water + phosphoric acid (1000-1 (volume / gm)) B. acetonitrile + phosphoric acid (1000-1 (v / dm)), The plan for mixing mobile phase solutions during the separation process during its passage through the separation column consists of - (20 min%), (15 30%, (25 min)) - 100%, (45 min0) % B = 0 and the average speed of the mobile phase. 1 ml / 1 min.

2.2. Approximate and qualitative chemical analysis of *S. officinalis*

Chemical analysis was performed according to qualitative analysis and chemical detection of some active substances in *S. officinalis*, based on the method of operation mentioned in Akrout, Mighri (12) and Aref, Bosal (13).

[1] Detection of Anthraquinones: 2 ml of chloroform CHCl₃ were added to 1 ml of *S. officinalis* extract in a test tube with shaking using filtration. Shake the filtrate again in the presence of an equal amount of 10% ammonia solution, and then a color appeared, followed by a Vortex mixer, a bright pink sign of the presence of Anthraquinones.
[2] Detection of flavonoids: In a test tube, an ammonia solution was added to the *S. officinalis* extract in a ratio of 1: 5, followed by the addition of 1 ml of concentrated sulfuric acid (H2SO4). The yellow color appeared then the indication of the presence of the flavonoids disappeared.

[3] Detection of glycosides: 5 ml of *S. officinalis* extract were added to 2 ml of acetic ice, followed by the addition of one drop of a solution of ferric chloride (FeCl3) and 1 ml of concentrated sulfuric acid. A brown ring formed on the inner face, indicating the presence of glycosides.

[4] Detection of Phenols: 5.0 ml of plant extract was placed in a test tube to which a few drops of a solution of ferric chloride (FeCl3) concentration of 5.0% were added, so that a dark green color was an indication of the presence of phenolic compounds.

[5] Detection of steroids. 2 ml of anhydrous acetate was added to 5.0 ml of plant extract, and then 2 ml of sulfuric acid (H2SO4) was added. The color changed from violet to blue or green indicating the presence of steroids.

[6] Detection of tannins. 5 ml of distilled water were added to 1 ml of plant extract and transferred to a water bath with a boil, then cooled the mixture with the addition of a few drops of a solution of ferric sulfate at a concentration of 1.0% gradually until a greenish-brown or bluish-black color appeared. As an indication of the presence of Tannins.

[7] Detection of Resins. 5 ml of hexane (C5H10) was added to 1.0 g of plant powder, followed by adding the same amount of copper acetate solution with shaking well, then leaving the mixture until the layers separated. A green color showed evidence of the presence of resins.

2.3. Animals used

The current study was conducted on male mice Balb/c, which were obtained from the animal house of the College of Veterinary Medicine / University of Tikrit, at an age of (8-10 weeks) months, with an average weight between (25-35) g.

2.3.1. Design of the experiment

The mice were distributed into randomly controlled and experimental groups, (40) adult male mice divided into 8 groups that included negative and positive control groups and 6
experimental groups by 3 groups for each of the aqueous and alcoholic extract respectively, as follows:

**First Group:** Includes five mice that were not exposed to CCl4 and were considered negative control.

**Second group:** Containof five mice that were exposed to CCl4 at a concentration of 2.3 mg / kg on the first day orally and were considered positive control.

**Third group:** Number of five mice that were exposed to CCl4 at a concentration of 2.3 mg / kg on the first day orally, then were given a solution of 100 mg / kg of aqueous extract after six hours and continued on a daily for one time for six days.

**Fourth group:** includes five mice that were exposed to CCl4 at a concentration of 2.3 mg / kg on the first day orally, and then given a solution of 200 mg / kg of aqueous extract after six hours and continued daily for one time for six days.

**Fifth group:** Number of five mice that were exposed to CCl4 at a concentration of 2.3 mg / kg orally on the first day, and then were given a solution of 300 mg / kg of aqueous extract after six hours and continued daily for one time for six days.

**Sixth group:** includes five mice that were exposed to CCl4 at a concentration of 2.3 mg / kg on the first day orally, and then were given a solution of 100 mg / kg of alcoholic extract after six hours and continued daily for one time for six days.

**Seventh group:** Containof five mice that were exposed to CCl4 at a concentration of 2.3 mg / kg on the first day orally, and then given a solution of 200 mg / kg of alcoholic extract after six hours and continued one time daily for six days.

**Eight Group:** Includes five mice that were exposed to CCl4 at a concentration of 2.3 mg / kg on the first day orally, then were given a solution of 300 mg / kg of alcoholic extract after six hours and continued one time daily for six days.

2.4. Collecting of blood samples

The blood was obtained from the mice from the corner of the eye by a capillary tube implanted in the sinus orbital (13). 2 ml of blood was placed in anticoagulant-free tubes. The serum was
separated by centrifugation at 3000 rpm for one minute, and stored in a freezer at -20 °C until the required analyzes were performed.(9).

2.5.Serum biochemical tests

Enzyme (AST) and (ALT) changes were assessed using a commercial analysis kit from the French company Biomerieux according to the Reitman and Frankel (13) based on colorimetric method, and enzyme changes (ALP) were assessed using an analysis same commercial kit according Kind and King (14) colorimetric method, as well as the enzyme (GST) using the Biolabo (French) kit, using the Hillman and Olt method (15).

3.6. Estimating the level of serum (MDA) in animals

Measurement of MAD, the product of the superoxidation of fats, was performed using the colorimetric method, which depends on the interaction between the atoruric acid and MAD according to the Al-Sephardard method (16).

2.7. Statistical analysis

The results obtained were analyzed using SAS 2001 program to find the significant difference between groups. Least Significant difference / LSD analysis was used at a probability level P≤ 0.05 to find the significant differences between levels of different treatments.

3. Results and Discussion

3.1. Measurement of the UV spectra

To confirm the separation of aqueous and alcoholic extracts of S. officinalis leaves using spectroscopy at 278, 356 nm for alcoholic extracts and 305 nm for aqueous extracts, the results are shown in Figure 1 (A and B).
Figure 1A. Uv-visible spectrum of alcoholic extract of *S. officinalis* leaves

Figure 1B. Uv-visible spectrum of *S. officinalis* leaves aqueous extract

### 3.2. High performance liquid chromatography (HPLC) analysis

Figure 2 shows the process of separating the compounds of the alcoholic extract of *S. officinalis* leaves, the appearance of the compound at a retention time of 3.2 minutes, in addition to other active compounds at a retention time of 7.6, 7.9 and 8.5 minutes by means of the HPLC high-performance liquid chromatography device (14).
3.3. Chemical detection of the active substances of the \textit{S. officinalis} extract

The chemical assessment of the active substances of the \textit{S. officinalis} extract, shown in table 1, the presence of flavonoids, saponins, steroids, tannins, terpenes, and alkaloids confirmed. In addition to other compounds in \textit{S. officinalis} extract, the function of tannins is due to the containing of some phenolic compounds such as callic acid and tannic acid, which has the ability to break down enzymes involved in amino acid production, that are essential in increasing cell division. As well as the action of saponins due to their containing saponin glycosides, as they have hydroxyl groups that have the ability to dissolve the lipid layer present in the cell walls, as it affects the selectivity of the cell wall, which facilitates the entry and exit of substances through the cell wall (15).

Elkhalafawy et. al (2015) showed that other secondary compounds with medicinal and physiological activity may act as anti-inflammatory and anti-allergic, anti-viral, anti-cancerous, and as powerful anti-oxidants and repellents to free radicals (16).

Table 1. The chemical analysis of the active substances of \textit{S. officinalis} leaves extract

<table>
<thead>
<tr>
<th>Seq</th>
<th>Materials</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Anthraquinones</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Phenols</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Saponins</td>
<td>+</td>
</tr>
</tbody>
</table>
3.4. GST enzyme activity

Figure 3 and the values in tables (2 and 3) show the effect of the aqueous and alcoholic extract of *S. officinalis* on GST enzyme in the blood serum of mice poisoned with carbon tetrachloride (at a concentration of 3.2 mg / kg).

The result showed that alcoholic extract has a higher effect than the aqueous, that's because the alcoholic extract contains a greater amount of active compounds such as flaviodins, terpenes and others. The findings indicated that, decreased level of GST activity in the group poisoned with 100 mg /kg of the alcoholic and aqueous extract of *S. officinalis*, compared with the positive control group, the value were 64.85 and 66.92U / dHb, respectively, and at a concentration of 200 mg / kg of both the alcoholic and aqueous extracts, significant decrease in the activity of GST enzyme was observed, the value was 58.76 and 61.37U / dHb, respectively. There was also the evidence of the effectiveness of the alcoholic and aqueous extract at a concentration of 300 mg / kg. The values are 53.19 and 57.09U / dHb, respectively.

According to the above, the enzyme’s activity decreased compared to the positive control group that was given CCl₄, because the effectiveness of GST enzyme increased with the administration of carbon tetrachloride because in treatment groups, the body contains antioxidants, thus helps to increase the body’s immunity (17).

From the above results, that the alcoholic extract of *S. officinalis* has the ability to increase the de-oxidizing substance, as a result reduces free radicals, thus the effect of the main substance for the function of GST enzyme decreased. Therefore, the effect of equal forms of GST transition coenzyme was observed in all hepatocellular carcinoma to control cell proliferation.(18).

There are many researches and reports that refer to different types of cancer, including esophageal and intestinal cancer, stomach cancer, and their effect on the activity of GST
enzyme, so it is necessary to maintain the balance of GST enzyme and also to preserve the contents or components of GST enzyme (reference).

Figure 3. The effect of the aqueous and alcoholic extract of *S. officinalis* on GST enzyme

### 3.5. Activity of ALP enzyme

The enzyme (ALP) is found in different tissue and organs of the body such as the intestine, bone marrow, liver and kidneys. Enzyme activity varied according to the change (pH), temperature and amount of acidity yield as well as the concentration of the main substances in the presence of activators or inhibitors in the reaction medium. (29) The results of the effect of the alcoholic and aqueous extract of *S. officinalis* at three concentrations of the ALP enzyme in the blood of laboratory mice poisoned with CCl4 and compared it with the negative control and positive control models were shown in figure 4 and tables 2 and 3. There were high significant differences (P≤0.05), ALP enzyme activity decreased in the group treated with 100 mg / kg of the alcoholic and aqueous extract compared with the group poisoned with carbon tetrachloride (65.80U / mg and 66.71U / mg, respectively). More decrease was observed in both groups treated with alcoholic and aqueous extracts at concentrations of 100 and 200 mg / kg. The activity of ALP in concentration of 300 mg / kg of the alcoholic or aqueous extract was 50.90U / mg and 54.06U / mg, respectively.
Figure 4. The effect of the aqueous and alcoholic extract of *S. officinalis* on ALP enzyme

Based on the results of this study, it is noticed that there is a significant effect of the alcoholic and aqueous extract of *S. officinalis* on the activity of the ALP enzyme in the blood of the laboratory mice poisoned with carbon tetrachloride compared control grope, which indicates that the cells had an antioxidant substance before CCl₄ was given as a stimulant. *S. officinalis* contains effective compounds that prevent enzymes from damaging of function and structure of proteins. It also inhibits the process of oxidation of cellular contents such as nucleic acids, proteins and lipids. (19).

### 3.6. AST enzyme activity

Aspartate aminotransferase (AST) (EC 2.6) enzyme is detected in high concentrations in many tissues such as the heart, liver and kidneys, and in lower concentrations in other tissues such as muscles, red blood cells, and other organ of the body (20).

Research and studies show that when damage occurs in the organs of the body, the concentration of AST enzyme in tissues increased. As in hepatitis, severe anemia, mononucleosis, hereditary hemorrhage, multiple tumors, postoperative, acute burns, primary muscle disease, and exposure to very dangerous pollutants such as C6H6 benzene, carbon tetrachloride CC14 the amount of enzyme in the tissue such as heart or liver increased (20).
Figure 5 and Tables (2 and 3) show the effect of aqueous and alcoholic extract of *S. officinalis* on serum AST enzyme of mice poisoned with carbon tetrachloride (at a concentration of 3.2 mg / kg). The results showed that the alcoholic extract has a higher effect than the aqueous extract, because the alcoholic extract contains a greater amount of active compounds such as flaviodins, terpenes and others. The results indicate a lower level of AST enzyme activity in the group treated with 100 mg / kg of the alcoholic and aqueous extract, the amount of enzyme in the serum of mice treated with concentrations of 100 and 200 mg / kg was 39.91 and 40.817 U / L, respectively. Also, the effectiveness of alcoholic and aqueous extracts at a concentration of 300 mg / kg was 36.23U / L and 38.32U / L, respectively.

The elevation of AST enzyme level increased with administration of serum carbon tetrachloride to the positive control group and this may indicate the severity of liver or other organ damage associated with CCl₄(21).

It is evident from the above results that the alcoholic extract of *S. officinalis* has the ability to reduce injury or damage to the liver or organs that may cause an increase in the value of AST.

### 3.7. Activity of ALT enzyme

Alanine aminotransferase (ALT) is the second aminotransferase enzyme formerly known as Glutamate pyruvate transaminase (GPT). This enzyme found in high quantities in the liver, and it is also detected in smaller quantities in the heart and skeletal muscle and others. ALT is
elevated in liver diseases, myocardial infarction, muscle diseases, and it rises when disorder occur in the bile duct, exercising, and exposed to toxic substances (20). The activity of the enzyme ALT increases significantly in the pathological conditions in liver tissue is affected with an increase in cell permeability due to the abundance of the enzyme in the cytoplasm (20).

Figure 6 and tables (2 and 3) showed the effect of the aqueous and alcoholic extract of S. officinalis on serum AST of mice poisoned with carbon tetrachloride (at a concentration of 3.2 mg / kg), it was observed that the alcoholic extract has a higher effect than the aqueous extract. The results indicate a lower level of activity of the enzyme ALT in the group treated with 100 mg / kg of alcoholic and aqueous extract of a S. officinalis compared with a control group (40.92 and 42.267U / L, respectively) and more decreased at a concentration of 200 mg / kg of both the alcoholic and aqueous extracts were 37.56 and 40.077U / L, respectively, while a significant decrease in the activity of the enzyme ALT was observed from each of the previous two groups. This is evidence of the effectiveness of the alcoholic and aqueous extract at a concentration of 300 mg / kg, where the value was 34.78U / L and 36.56U / L, respectively.

![Figure 6. The effect of the aqueous and alcoholic extract of S. officinalis on the ALT enzyme](image)

The elevation of the enzyme ALT level increased with administration of serum carbon tetrachloride to the positive control group. This may indicate the severity of liver or other organ damage associated with CCl₄ (21). According the results of this study the alcoholic extract of S. officinalis has the ability to reduce injury or damage to the liver or organs that may cause an increase in the value of ALT.

3.8. The level of malondialdehyde (MDA) activity
The results of study showed that the effect of aqueous and alcoholic extract of *S. officinalis* at three different concentrations on the level malondialdehyde activity (MDA) in the serum of laboratory mice after poisoned with carbon tetrachloride (at a concentration of 3.2 mg / kg, for one day) with high significant differences compared with the negative and positive control groups. Results in figure 7 and tables (2, 3), indicated that decrease in MDA in the level of lipid peroxidation in the group treated with 100 mg / kg of extract compared with negative control group. Either in the group treated with 200 mg / kg of extract, whether aqueous or alcoholic, a decrease was observed more than a group of 100 mg / kg and this is evidence of the effectiveness of the extract.

![Graph showing MDA activity](image)

**Figure 7.** The effect of the aqueous and alcoholic extract of *S. officinalis* on the MDA activity

A significant decrease was observed in the group treated with 300 mg / kg of extract compared to the previous two groups. This indicates the effect of increasing the concentration of the alcoholic or aqueous extract of *S. officinalis* on the activity and effectiveness of lipid peroxidation (22). The alcoholic or aqueous extract of *S. officinalis* was effective after toxicity of CCl4, which indicates that the cells contained the antioxidant flavonoids. Treatment of laboratory animals with alcoholic or aqueous extracts of *S. officinalis* leads to a decrease in the level of MDA, which confirms its role as an antioxidant because it contains active substances that have a high potential and effectiveness in reducing free radicals by a significant rate (23).

**Table 2.** Effect of alcoholic extract of *S. officinalis* on biochemical parameters levels in mice poisoned by CCl4 (*P* ≤ 0.05)
Data as mean ± SE. (n = 5).

Table 3. Effect of aqueous extract of *S. officinalis* on biochemical parameters levels in mice poisoned by CCl₄ (P ≤ 0.05)

<table>
<thead>
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<th>Parameter</th>
<th>negative control</th>
<th>positive control</th>
<th>alcoholic extract</th>
</tr>
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<td></td>
<td></td>
<td></td>
<td>100mg/Kg</td>
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<tr>
<td>ALP</td>
<td>45.63±3.67</td>
<td>69.32±4.01</td>
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<tr>
<td>MDA</td>
<td>11.68±1.27</td>
<td>30.04±2.12</td>
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<tr>
<td>GST</td>
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<td>2.2±0.09</td>
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<tr>
<td>ALT</td>
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<tr>
<td>AST</td>
<td>39.06±4.78</td>
<td>46.31±3.56</td>
<td>42.12±2.89</td>
</tr>
</tbody>
</table>

Data as mean ± SE. (n = 5).

The results of the study showed that the high concentrations of the alcoholic or aqueous extract of *S. officinalis* have antioxidant effect. It showed that the alcoholic extract has a higher effect than the aqueous extract, because the alcoholic extract contains a greater amount of active compounds such as flavonoids, terpenes, and others, as well as main active substances to control the effectiveness of all the studied enzymes in the laboratory animal blood serum after administration of the inducer of carbon tetrachloride.

References