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A Study of The Effect of Aqueous and Alcoholic Extract of Saliva on The Reduction of Induced Hepatotoxicity in Laboratory Animals with Carbon Tetrachloride CCL4

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Abstract

The study aimed to know the effect of the aqueous and alcoholic extract of the Saliva plant on laboratory rats in which poisoning with carbon tetrachloride was introduced. It was divided into four groups. The first group represented the healthy control. Water and food were only given, while the disease- infected control was injected with protonate at a rate of 1 ml / kg / day for four weeks.

The third and fourth group injected protons with 1 ml / kg / day and an oral dose of 1 ml / kg / day of aqueous and alcoholic extract, respectively, for a period of four weeks. Biochemical tests were performed, urea and creatinine were measured, and there was a significant decrease in the disease- infected control compared to the healthy control and an increase compared to the aqueous and alcoholic extract group at a probability level of 0.05.

As for the total protein, uric acid, TC, TG, HDL, LDL, AST, ALT, a significant decrease was found in the aqueous and alcoholic extract groups at the probability level 0.05 compared to the infected control.

Keywords: aqueous and alcoholic extract of Saliva, carbon tetrachloride, biochemical tests, histological studies.

Introduction

The liver, which is the main organ for drug metabolism, is itself the main place for drug diseases.

Drug-induced liver disease remains a problem for many pharmacological uses, It is a major challenge to identify possible treatments as well as alcohol-related liver diseases which are the main cause of chronic liver disease [(1)] And it leads to an increase in mortality rates around the world due to imbalances that occur in the delicate balance between oxidants and antioxidants, leading to oxidative stress, represented by an increase in the production of lipid peroxide and changes in the composition and functions of important cellular components such as proteins and DNA [(2)]. Sage belongs to the Labiatae family and is considered one of the oldest medicinal herbs. The name Saliva comes from the Latin word Salvare, which means to save, meaning to preserve life, and fficinalis, which means medicinal, referring to the medicinal properties that this plant possesses, and the plant height is about 60 cm It has greenish-silver-colored leaves, and its flowers are of different colors between white, blue and pink, and the plant blooms in the spring [(3)] The plant is known by other names such as Al-Quwaisa Al-Makhzaneia and Al-Kesaen in Arabic [(4)].

The Saliva plant grows naturally and is cultivated in Iraq and other regions in the Middle East and different regions of the world, especially in Turkey, Albania, Yugoslavia, Greece and Jordan. There are about 900 species of *Salvia* (Sage) , but only a few types of it are commercially important and *Salvia officinalis* is one of the most important species due to It contains most of the active compounds present in the rest of the other species [(5)].

The active ingredients differ according to the plant part used (such as the roots or the aerial parts such as the

leaves and flowers). Saliva leaves are rich in flavonoids, which are of important biological activity produced by the plant to protect its parts from the damage of bacterial and fungal diseases. For the same reason, they can be considered natural biocides produced in the plant in response to exposure to Pathogens [3] and other active ingredients are phenols and terpenes [5] In addition, Saliva leaves contain vitamins, including E, C, A, B-Complex and essential oils, as well as tannins and saponins. [6] As for the medical fields, it is used in the treatment of stomach and mouth ulcers, gum and mouth infections, high body temperature, diarrhea, and kidney and intestinal disorders. [3 , 4] It is also a digestive stimulant and helps in calming the nervous system and regulating body hormones, especially estrogens [5], as well as fighting oxidative free radicals [5].

Methods and Materials

The Saliva plant was obtained from a herbarium in the city of Samarra, where it was ground with an electric grinder and placed in polyethylene bags until use.

1- Finding the nutritional values of Saliva plant, including:

1-1- Humidity: The humidity in a convection oven was estimated at a temperature of (130) for an hour and until the stability of weight (7)

1-2-Humidity percentage = (Dry Before Weighing) / (Dry After Weighing) x 100

1-3-Flavonoids: 10 gm of the substance is taken and added to 100 ml of ethanol at a concentration of 80% and filtered with a filter paper and the percentage of flavonoids are calculated (8).

% Of flavonoids = (flavonoids weight) / (substance weight) x 100

1-4- Ash: Ash was estimated in 2 g of the sample according to (7)

% Of ash = (Ash Weight) / (Sample Weight) x 100

1-5- Fibers: The fibers were determined according to (7).

Fiber percentage = (fiber weight) / (sample weight) x 100

2- Preparation of the aqueous extract of Saliva

The aqueous extract was prepared by soaking 50 g per 200 ml of water at a temperature of 70 ° C and left for 24 hours, after which it was filtered to obtain the extract, then it was dried by a drying oven and the extract was obtained and kept in cooling until use.

2- Preparing the alcoholic extract

Use 50 grams of sage in 200 ml of (70% ethyl alcohol) and put it on a magnetic mixture for 10 minutes at a temperature of 40 ° C, then after that it was left for 24 hours at room temperature in a dark place and filtered out the extract and repeated the process for more than three time And then it was dried by the rotary evaporator at a temperature of no more than 45 ° C and the extract was kept until use

The percentage of the extracted material in the aqueous and alcoholic extract was calculated through the parameter (9).

Percentage of extracted material = (extract material weight) / (sample weight) x 100

3- Detect the effective ingredient

The active ingredient and aggregates found in the sage plant have been detected and include detection of phenols, flavonoids, saponins, tannins, alkaloids and glycosides (10).

Experimental animals

In this study, white rats were used, with a number of 20 for a period of two weeks, weighing (200-220 g) and divided equally by 5 animals per group, and it was obtained from the General Company for Medical Supplies in Samarra.

Group I: the control group (C_1), were given water and food only

Group I: infected control group C_2 injected protonate 1 ml / kg / day of CCL_4 for four weeks.

Group III: G1, injected protonate of 1 ml / kg / day of CCL_4 and an oral dose of 1 ml of aqueous extract of the Saliva plant for four weeks

Group III: G2, injected protonate of 1 ml / kg / day of CCL_4 and an oral dose of 1 ml of the alcoholic extract of the Saliva plant for four weeks.

The treatment was done orally using the Gavage Needle tube and the experiment lasted for four weeks, and after the end of the injection, the animals were killed and blood was taken from them.

Prepare blood samples

Blood samples were collected and then separated by means of a centrifuge at 3000 rpm for 15 minutes. Serum was obtained and kept in the freezing until use.

Biochemical tests

The concentration of total protein, urea, creatinine, albumin, uric acid, ALP, AST, cholesterol and triglycerides were measured using the ready-made kit from Bio Maghreb.

Results and Discussion

Table 1. shows the nutritional values of the sage plant according to the table

Percentage	Nutritional values	No
7.37%	Humidity	1-
92.62%	Total solids	2-
5.61%	Ash	3-
9.72%	Fiber	4-
8.7%	Flavonoids	5-

Through the above table, it was found that the percentage of moisture was 7.37%, as for the total solids, it was recorded at 92.62%, as for the ash, it was recorded at 5.61%, and the fibers were recorded at 9.72%, while the flavonoids were recorded at 8.7%.

Table 2. shows the percentage of the extracted material (remaining)

percentage	Abstract	No
24%	Aqueous extract	1-
35%	Alcoholic extract	2-

The results of the qualitative detection indicated the containment of some active substances in the Saliva plant,

as it became clear from Table (1) that the aqueous extract of Saliva contains flavonoids, phenols, tannin and saponins, while the alcoholic extract of Saliva contained alkaloids, flavonoids, phenols, terpenes and glycosides, as shown in the table.

It is evident from the below table that the aqueous extract of Saliva contains flavonoids, phenols, tannins, terpenes, and saponins, while no color detection of alkaloid and glycoside was shown.

As for the alcoholic extract, it was shown to contain alkaloids, flavonoids, phenols, terpenes, and glycosides, while tannins and saponins did not appear.

The results of the study agree with (Raghad et al.) (11) where the aqueous and alcoholic extract mixed in the leaves and stems of the Saliva plant contain glycosides, flavonoids, resins, saponins, terpenes, alkaloids and phenols, as well as the study agrees with (Al-Obaidi et al.) (12) Whereas, the mixed aqueous extract , alcoholic extract and acetic acid contains flavonoids, glycosides, phenols, terpenes, saponins, alkaloid and flavonoids.

Table 3. shows the qualitative statements of the aqueous and alcoholic extract in the sage plant

Alcoholic extract	Aqueous extract	The change	Reagent type	item	No
+	-	Orange color	Dargduf	Alkaloids	1
+	+	yellow color	Ammonia solution	Flavonoids	2
-	+	Light brown precipitate	Lead acetate	Tannins	3
+	+	Bluish green color	Potassium ferrocyanide	Phenols	4
+	+	Dark red	Salkovsky	Terpenes	5
_	+	White precipitate	Mercuric chloride	saponins	6
+	_	Red precipitate	Benedict's reagent	Glycosides	7

Biochemical tests

Table 2. shows the rate and standard deviation of the parameters under study

P-Value	The Group				Para meter
	G2	G1	C2	C1	
	Mean±S.D N=60				
≤0.05	34.26±5.55	28.02±2.51	37.06±5.26	25.2±2.8	Urea (mg/dl)
≤0.05	0.6740±0.509	0.6748±0.1338	1.23±0.2	0.8654±0.1766	Creatinine (mg/dl)
≤0.05	.302±0.877	4.842±1.5514	6.188±1.081	7.2±0.62	Total Protine (mg/dl
≤0.05	0.5003±0.1086	0.4800±0.1672	0.5700±0.128	0.61±0.13	Uric acid (mg/dl)
≤0.05	3.170±0.504	3.560±0.527	2.866±0.407	4.5±0.56	Albumine (mg/dl)
≤0.05	2.26	1.28	1.5	2.7	Glub. (mg/dl)
≤0.05	82.24±4.52	92.24±1.32	164.08±10.01	150.75±3.2	c-cholestrol (mg/dl)
≤0.05	130.34±2.9	155.78±2.1	180.08±2.9	216±0.22	Triglyceride (mg/dl)
0.05	39.2±0.6	42.40±1.3	46.3±2.9	38.12±2.1	HDL (mg/dl)
≤0.05	49.61±0.4	54.34±2.3	87.3±4.2	72.2±8.2	LDL (mg/dl)
≤0.05	26.06±0.58	31.156±0.42	36.01±0.05	43.2±0.044	VLDL (mg/dl)
≤0.05	60.50±1.02	65.30±2.3	75.80±1.5	53.21±2.4	AST U/L
≤0.05	43.2±0.44	55.20±0.9	79.1±1.2	38.03±0.2	ALT U/L

The reason for using carbon tetrachloride is to induce toxicity in the animal's liver [13] carbon tetrachloride is metabolized by hepatic microsomal CYPs to the toxic metabolites of the liver are chloroform (CCl3) and trichloromethyl peroxyl (CCl3OO) [14] are unstable roots and show strong affinity to bind to protein and fats from the cell membrane or to extract hydrogen atom from unsaturated fats, which leads to lipid stimulation and oxidation and causing liver damage [15].

Through Table No. (2) it was found that the increase in urea and creatinine in the affected control compared to the healthy control and the reason for that is according to what is mentioned in (15)

Table No. (2) shows high total cholesterol, LDL, HDL, and liver enzymes in the affected control compared to the healthy control, and this corresponds to (Yasmine et al.) [16] because the liver damage caused by carbon tetrachloride is closely related to metabolic activation. For short-lived reactive media, in which the halogens of carbon tetrachloride are catalyzed by cytochrome P450, a terminal oxidase of the hepatic mixed function oxidase system.

When carbon tetrachloride is used to induce cell damage, it can either result from the covalent binding of the reactive media with the cellular components, or from the enhanced lipid peroxide resulting from the interaction of the free radial media with oxygen which in turn will attack the unsaturated fatty acids. This entire procedure destroys lipids, especially unsaturated phospholipids leading to damage to the intracellular and plasma membranes [17].

It is evident from the above table that the aqueous and alcoholic extract of sage led to a significant decrease in the level of total cholesterol, HDL, LDL, and liver enzymes compared to the affected control, and the reason for this is that the Saliva plant contains flavonoids, phenol compounds and quercetin, which are antioxidants [18] The results of the present study agree with (Saeed et al.) [19], and it was also found that the aqueous extract of the Saliva plant inhibits the production of dialdehyde-melone in the brain and liver of mice, and it was found that the sage extract leads to a significant increase in glutathione-S-transferase and the reduction of liver glutathione in mice. [20][21] It also reduces the release of liver enzymes from hepatocytes and reduces their levels in the blood [20][22]

Histological study

Effects on the histology of the livers

Group 1: The histological examination of the livers of rats

in the control group under a light microscope showed the normal shape of the bronchial tissue, the hepatocytes were normal and arranged as Cords and the presence of Sinusoids and Centeral vein.

Group 2: Treatment with carbon tetrachloride resulted in degenerative changes in composition Histopathology of affected rat livers marked by Sinusoids expansion and necrosis Hepatocytes and Infiltration of Inflammatory Cells and Irregular Cords Hepatobiliary.

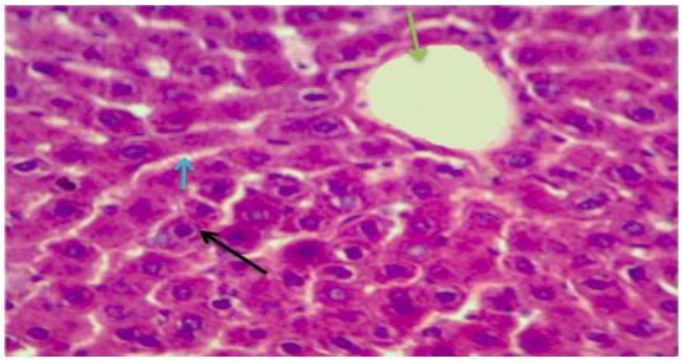


Figure 1. A ra t-liver segment from a control group, showing an arrangement Hepatic cords(black arrow), Sinusoids(blue arrow) central blood vessel(yellow arrow 400X: H&E.

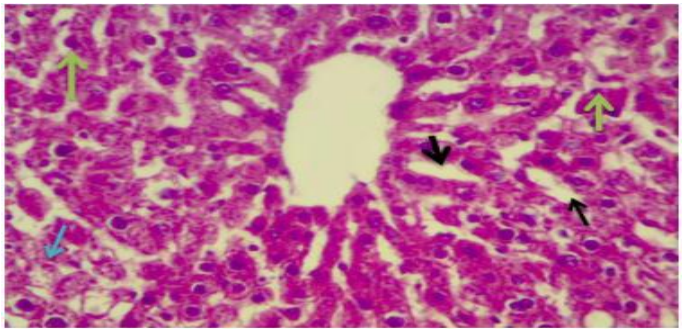


Figure 2. Control infected with carbon tetrachloride, showing expansion Sinusoids (black arrow), Necrosis of hepatocytes (blue arrow) Irregular Cords Hepatobiliary (yellow arrow) 400X: H&E.

Group 3: Show treated histological sections With alcohol extract of Saliva, back the histological structure, hepatocytes, lymphocytes, migrating cells, dividing cells A simple distribution in the Sinusoids

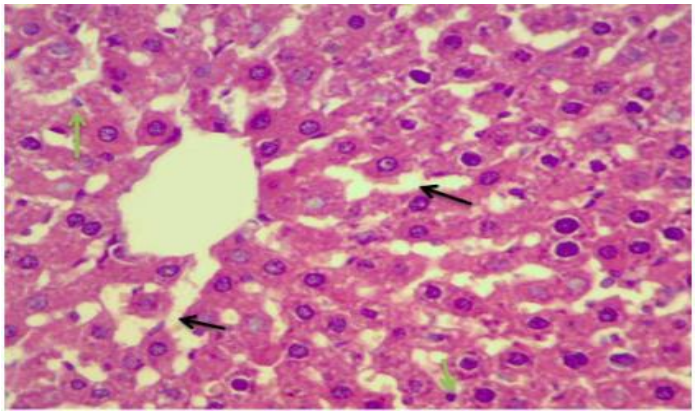
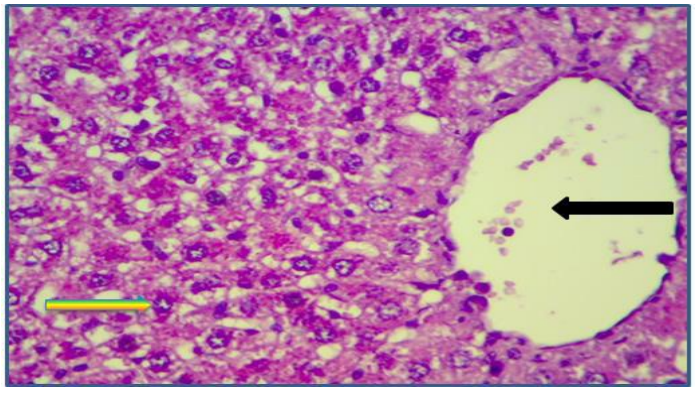


Figure 3. expansion Sinusoids (black arrow), Migrating lymphocytes (yellow arrow) 400X: H&E.

Group 4: Show treated histological sections With Aqueous extract of Saliva, The study showed the presence of congestion and minimal dissection between hepatocytes

Figure 4. Median vein (black arrow), Hepatocytes (yellow arrow) 400X: H&E



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