The protective effect of the alcoholic extract Lactuca serriola on some physiological parameters in the liver in white mice induced with Benzo(a)pyrene

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Abstract
This study was conducted to investigate the possibility of using the alcoholic extract Lactuca serriola as a protective agent against the chemical compound benzopyrene that causes cancer. Alkanes are the dominant group in addition to the presence of phenol, alkene and ester, the highest concentration of the compound (1-Henicosyl formate) (42.579) and the lowest concentration of the compound (1,6-Octadien-3-ol, 3,7-dimethyl-, 2- aminobenzoate) by (1.009%), the results showed a significant decrease at the level of probability (P≥ 0.05) in the level of total protein in the liver in the group of animals that were dosed with carcinogenic benzopyrene in comparison with the healthy control group, while the effect on the level of total protein was less than in the group of animals that were dosed with carcinogenic benzopyrene, while the effect on the level of total protein was less than in the healthy control group. The groups that were dosed with alcoholic extract of wild lettuce plant with a concentration of 0.006mg/kg for the first group, 0.003mg/kg for the second group, and a concentration of 0.0015mg/kg for the third group in conjunction with the benzene and pyrene carcinogen in comparison with the control group. It also showed a significant increase at the level of probability (0.05≥P) in the activity of the transaminase enzymes AST and ALT in the liver in the group of animals that were dosed with the carcinogenic substance benzopyrene compared to the healthy control group, while the effect on the level of enzymes was less in the groups that were dosed with alcoholic extract of wild lettuce plant with a concentration of 0.006mg/kg for the first group and 0.003mg/kg for the second group and 0.0015mg/kg for the third group in conjunction with the carcinogenic benzopyrene and compared with the control group.

Keywords: Protective, Effect, Induced, Parameters.

INTRODUCTION
The liver is the largest glandular organ in the human body and is divided into four lobes of unequal size. It is located on the right side of the abdominal cavity below the diaphragm (Jahangir, 2011) up to The blood is delivered to it by the hepatic artery, which carries oxygen, and the portal vein, which carries it Blood loaded with digested food from the intestines, as the liver plays a major role in metabolism and a number of Other body functions such as glycogen storage, the liver is also a manufacturing center Proteins, given the vital functions of the liver, a person may die within 24 hours of stopping Liver Action (Robin & Rachel, 2018)

Antioxidants are special defenses that the body has that act on Protecting it from damage caused by free radicals, as studies have shown that antioxidants work To reduce the risk of diseases, the most important of which are heart disease and atherosclerosis Cataracts, aging, cancer, diabetes, and liver disease (Rajendran et al., 2014). The main and primary role that antioxidants of various types play is their work on It donates an electron to the free radical for the purpose of preventing its interactions and stopping its disruptive activity, as well as working to Decomposition of peroxide, some of which are metal chelating materials (Rahal et al., 2014)

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agent, and it is either soluble in water or soluble in fat, as mentioned by (Nimse and Pal, 2015) Medicinal plants and the active substances that are extracted from them play a major role in the treatment of many diseases Diseases, the most important of which is the wild lettuce plant L.serriola, which has proven effective in protecting the liver against The causes that lead to liver tissue damage, as it is rich in antioxidants that reduce infection with diseases has proven effective in liver tissue caused by high blood sugar level (Kailash et al., 2020) Or the increase may be due to hepatocyte hypertrophy due to the drugs and thus stimulating the network endoplasmic reticulum to produce more enzymes in proportion to the size of the cell (MacSween & Whaley, 1992, or resulting from the breakdown of hepatocytes, leading to the release of enzymes into Circulation (Khan et al., 1999). The aim of the study was to test the effect of wild lettuce as a preventative against cancer and its effect on some physiological aspects of the liver.

**Materials And Methods**

L. serriola wild lettuce leaves was collected from separate areas in Fallujah district on 6/2021, where the accepted collection rules were followed, where samples were collected in the afternoon because they were saturated with sunlight (Ruwaiha, 1983), and the parts were taken fresh, healthy, and free from diseases or ruptures, after it was fully ascertained that the plant had completely dried, it was ground using the electric grinder, and then the plant powder was placed inside filter papers to prevent exposure to the effects of moisture, and then it was kept inside glass containers, (Al-Hashemi, 2005).

**Gas chromatography-mass spectrometry GC-MS analysis**

The filtered and alcoholic extracted plant samples are placed in the GC-MS device of the type GC-MS-QP2010 Plus (Shimadzu, Japan), helium gas (purity) 9, used to carry the gas at a constant flow rate of 1 ml (1m) per minute, either the degree of The temperature of the column starts from 80 °C, and gradually increases every ten degrees 10 °C until it reaches 280 °C, while the temperature of the heat source of the device reaches 350 °C, the initial temperature of the device was set at 80 °C while maintaining this temperature for a period Two minutes, and at the end of this period, the temperature of the Oven is raised to 280 °C, with an increase of 5 °C per minute, and it remains for 9 minutes. As for the temperature of the injection port, it remains within 280 °C, and the flow rate of helium gas is 1 ml per minute, and the value of the ionization energy is 70 ev (Electron volt, then the separation is accomplished at a column temperature of Column 5 MS for 30 minutes. As for the Quadrupole mass detector, it was used to detect compounds through an opening in the column, the temperature of the detector reached detector 280 °C and the chemical compounds of plant samples were compared. The chemical compounds stored in the computer library type Nist linked with the GC-MS Ghafar et al., (2010) biological experiment Soak 4 g of plant powder in 100 ml of methyl alcohol at a concentration of 99% for 24 hours in a vibrating tabletop incubator. After that, the alcohol is evaporated at a temperature of 37 °C by leaving the extract in the incubator for 48 hours, then the extract is suspended with 10 ml of distilled water, sterilized and kept at 20 °C until it is time for use, using Benzo(a)pyrene From (Guokang (China) in the development of cancer in experimental animals, and since it is a solid insoluble in water, it was dissolved in vegetable corn oil according to the method (Wattenberg, 1993 & Richard). Experience design, 35 adult white male mice were used in this study, which were brought from the National Center for Drug Control in Baghdad, they were placed in plastic cages for animal husbandry, which were spread with sawdust, taking care to clean the cages and changing the sawdust at a rate of three times a week. Mice in cages by 7 mice in one cage, while the number of cages was 5 cages. The mice were subjected to appropriate laboratory conditions in terms of ventilation, temperature and appropriate lighting, while they were given water continuously, and fed with the standard diet.

**Laboratory mice were divided into 5 groups as follows**

The negative control group that was given water only, the positive control group that was given benzo(a)pyrene orally at a concentration of 0.001 mmol and one dose per week, while the groups of the extract of wild lettuce L. serriola were distributed according to the concentration of the extract To three groups, the group that was dosed with the extract with a concentration of 0.006 mmol, the group that was dosed with the extract with a concentration of 0.003 mmol, and the group that was dosed with the extract with a concentration of 0.0015 mmol on a daily basis for 30 days.

**Biochemical tests**

The animals were sacrificed after the end of the prescribed treatment period, and according to the McKenzie method, (2011), they were anesthetized using chloroform, then the animals were dissected and the livers were removed from two animals from each group for the purpose of biochemical study, where they were placed directly in the ice for the purpose of preserving the vitality of the liver.

1 - Estimation of the effectiveness of Total protein

The amount of total protein in the tissue extract of the liver was estimated using the Biuret method method (Falkner & Meites, 1982), as this method depends on the interaction of copper ions Cu + 2 in the basic medium with peptide bonds, and thus the production of a complex compound with a violet color as The resulting color intensity is proportional to the protein concentration in the sample.

2 - Determination of the activity of the aminotransferase enzymes AST and ALT, According to the method (Schmidt, 1963) (Reitman and Frankel, 1957), the activity of AST and ALT enzymes was measured.
Statistical Analysis

The data were statistically analyzed according to the Complete Randomized Design (CRD) model using the statistical program GenStat- Tenth Edition Version 10.3.0.0, and the significant differences of the means were tested using the least significant difference test L.S.D at the probability level (P≤0.05) (Al-Rawi and Khalaf Allah, 1980), in addition to using Microsoft Word Excel 2016.

RESULTS AND DISCUSSION

Chemical analysis by GC_MS of the raw alcoholic extract of the wild lettuce plant L. serriola revealed the presence of 12 compounds as in Table (1), which were detected using Gas Chromatography Mass Spectrometry (GC-MS) for chemical classification that helps in diagnosing and isolating the active compounds in the genus, it was noted that the abundance of the chemical content in terms of quantity and quality.

### Table 1. Chemical detection by GC_MS of the crude alcoholic extract of L. serriola

<table>
<thead>
<tr>
<th>Compound</th>
<th>Total % of</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Undecane, 2,4-dimethyl</td>
<td>1.022%</td>
<td><img src="structure" alt="Undecane" /></td>
</tr>
<tr>
<td>Silane, cyclohexyldimethoxymethyl</td>
<td>2.561%</td>
<td><img src="structure" alt="Silane" /></td>
</tr>
<tr>
<td>Dodecane</td>
<td>5.557%</td>
<td><img src="structure" alt="Dodecane" /></td>
</tr>
<tr>
<td>1,6-Octadien-3-ol, 3,7-dimethyl-2-aminobenzoate</td>
<td>1.009%</td>
<td><img src="structure" alt="Octadien" /></td>
</tr>
<tr>
<td>Hexadecane</td>
<td>1.431%</td>
<td><img src="structure" alt="Hexadecane" /></td>
</tr>
<tr>
<td>3-Cyclohexene-1-methanol, .alpha,.alpha,.4-trimethyl-, acetate</td>
<td>11.672%</td>
<td><img src="structure" alt="Cyclohexene" /></td>
</tr>
<tr>
<td>Tetradecane</td>
<td>4.956%</td>
<td><img src="structure" alt="Tetradecane" /></td>
</tr>
<tr>
<td>Phenol, 2,4-bis(1,1-dimethylethyl)-</td>
<td>21.644%</td>
<td><img src="structure" alt="Phenol" /></td>
</tr>
<tr>
<td>Hexadecane</td>
<td>2.079%</td>
<td><img src="structure" alt="Hexadecane" /></td>
</tr>
<tr>
<td>7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione</td>
<td>3.049%</td>
<td><img src="structure" alt="Oxaspiro" /></td>
</tr>
<tr>
<td>1,2-Benzenedicarboxylic acid, diisooctyl ester</td>
<td>2.443%</td>
<td><img src="structure" alt="Benzenedicarboxylic" /></td>
</tr>
<tr>
<td>1-Heneicosyl formate</td>
<td>42.579%</td>
<td><img src="structure" alt="Heneicosyl" /></td>
</tr>
</tbody>
</table>
The study showed that the compound 1-Heneicosyl formate had the highest ratio, reaching 42.579%, compared to the compound 1,6-Octadien-3-ol, 3,7-dimethyl-2-aminobenzoate, which was the lowest with a percentage of 1.009%, and this is what was done Illustrated in Table (1) where the alcoholic extract contains saturated alkanes, phenols, alkene, fatty acids, inorganic compounds and esters, as the alkanes appeared more dominantly than the rest of the compounds, as this biological division leads to the identification of cancer-inhibiting chemical compounds as they contain On each of 1,2-Benzenedicarboxylic acid, diisooctyl ester, Hexadecane, 1,6-Octadien-3-ol, 3,7-dimethyl-2-aminobenzoate, (Phenol,2,4-bis(1,1-dimethylethyl, Silane, cyclohexylidimethoxymethyl. Total protein in the Liver

The results of the biological study showed a significant decrease at the probability level (0.05≥P) in the rate of total protein in the liver of the group of animals exposed to the carcinogen benzo(a)pyrene compared with the healthy control group, where the rate of decrease was 3.06 Fig. 1. As for the three groups that were dosed with the alcoholic extract of L. serriola as a preventive and reference measure in conjunction with giving them the carcinogen Benzo(a)pyrene, and when compared with the healthy control group, the results were less effective, as the results of the first group showed a concentration of 0.006mml of Alcoholic extract of wild lettuce plant and the substance What is taken into consideration is that what was affected by the results of the control group, as the results of the second group amounted to a concentration of 0.003mml of influences, influences, influences, influences, influences, influences, influences, influences, influences. The control group, the results of the third group (concentration 0.0015mml)) with the control group, the last figure (1).

![Figure 1: Protective effect of alcoholic extract of L. serriola in total protein concentration in the liver of laboratory albino rats exposed to the carcinogen Benzo(a)pyrene.](image)

The effect of benzopyrene in decreasing the average concentration of total protein in the liver may be caused by damage and inflammation of the liver through its effect on the plasma membrane of hepatocytes. He agrees with (Kolade & Oladiji, 2018) about the toxic effect of benzopyrene on the decrease in the total protein concentration resulting from the effect of benzopyrene on the liver, as it destroys it functionally and structurally. Oxidative stress (Loekle et al. 1983), while not consistent with that of Seong et al. (2008) who found that exposure to B(a)p benzopyrene had no significant effect on total protein concentration in rockfish Sebastes schlegeli. The effect was less when animals were dosed with alcoholic extract of wild lettuce plant in conjunction with giving them the carcinogen. Enzymes into the bloodstream and works to curb free radicals and reduce oxidative damage and this was confirmed by (Asif et al., 2020).

Alanine aminotransferase (ALT)

The results of the biological study showed a significant increase at the probability level (0.05≥P) in the liver ALT enzyme level in the group of animals that were orally dosed with the carcinogen Benzo(a)pyrene compared with the healthy control group, where the rate of increase in the enzyme level was 142.39 Figure 2. The three groups that were orally dosed with alcoholic extract of wild lettuce plant L. serriola as a preventive and reference measure in conjunction with the carcinogen benzo(a)pyrene and compared to the healthy control group showed a lower effect on the enzyme level, where the results of the first group with concentration 0.006mml showed From the alcoholic extract of wild lettuce plant in conjunction with giving it the carcinogenic effect less than if the carcinogen was given alone, where it amounted to 100.56) compared to the control.
group, which is the effect closest to the natural group (Fig. 2), and the results of the second group with concentration 0.003 mmol of alcoholic extract of wild lettuce plant in conjunction with giving the carcinogenic substance less effects than if the carcinogen was given alone, where the average effect was 110.40, compared with the healthy control group (Fig. 2). The alcoholic effect of wild lettuce and the carcinogen was less than if the carcinogen was given alone, where the average effect was 121.22, compared to the healthy control group Fig. 2.

**Figure 2**: Protective effect of alcoholic extract of *L. serriola* in ALT alanine concentration in male laboratory mice exposed to the carcinogen benzo(a)pyrene.

Benzopyrene had a significant effect on the increase in the level of alanine enzyme ALT in animals that were orally dosed with the carcinogenic substance. She indicated that the cause of the increase is due to the toxic effects of benzopyrene on the liver, which are represented by its active metabolites, oxides, hydroxyl and polyhydroxylation, where these activated radicals covalently bind to large molecules and induce peroxidative decomposition of membrane lipids of cell membranes, mitochondria, lysosomes, and the endoplasmic reticulum rich in multiple fatty acids. This unsaturated lead to the formation of lipid peroxides, as peroxidative degradation of biofilm lipids is one of the main causes of hepatotoxicity of benzopyrene.

The current results also agreed with (Sherif et al., 2020), which indicated a significant increase in the level of ALT, which is the most specific enzyme for the liver and therefore is the best factor for detecting liver injury, and high levels of the enzyme indicate cellular leakage and Loss of functional integrity of cell membranes in the liver.

When animals were dosed with the alcoholic extract of the wild lettuce plant in conjunction with giving them the carcinogenic substance, the effect on the level of the enzyme was less than if the carcinogen was given alone. The current results showed that the alcoholic extract of the wild lettuce plant has a protective role for the liver against chemicals. The alcoholic extract of the wild lettuce plant maintains the cell membrane of hepatocytes and thus reduces the leakage of enzymes into the bloodstream and works to curb free radicals and reduce oxidative damage, and this was confirmed by (Asif et al., 2020).

**Aspartate aminotransferase (AST)**

The results of the current study showed a significant increase in the probability level (0.05≥P) in the level of liver AST in the group of animals that were orally dosed with the carcinogen Benzo(a)pyrene in comparison with the healthy control group, where the rate of increase in the enzyme level was 170.54  Figure 3. The results of the three groups that were orally dosed with alcoholic extract of wild lettuce plant *L. serriola* as a preventive and reference measure in conjunction with the carcinogen benzo(a)pyrene and compared with the healthy control group, showed a lower effect in the level of AST enzyme, where the results showed The first group with a concentration of 0.006mmol of alcoholic extract of wild lettuce plant in conjunction with the carcinogenic substance had a lower effect on the level of AST enzyme compared to if the carcinogen was given alone, where the average increase in the level of the enzyme was 133.90) compared to the control group, which is the closest effect to The normal group is Figure (3), and the results of the second group with a concentration of 0.003mmol of alcoholic extract of wild lettuce plant simultaneous with giving it
Benzopyrene had a significant effect on the increase in the level of AST enzyme in animals that were orally dosed with the carcinogenic substance. The flow of the enzyme into the blood circulation, and this causes an increase in the levels of the enzyme. These results are consistent with Seong et al., (2008)), which indicated a significant increase in the level of AST, and that this rise is caused by liver damage due to the toxic effects it causes. Benzopyrene in the liver, where high levels of AST transporter enzyme indicate liver damage such as that caused by viral hepatitis, as well as myocardial infarction and muscle injury, where high levels of enzymes indicate cell leakage and loss of functional integrity of the hepatocyte membrane.

It also agrees with the results of (Benchouieb et al., 2019), which indicated that the group treated with benzopyrene showed a very significant increase in AST enzyme levels compared to the control group.

When animals were dosed with alcoholic extract of wild lettuce plant in conjunction with giving them the carcinogen, the effect on the level of AST transporter enzyme was less than if the carcinogen was given alone. The current results showed that the alcoholic extract of wild lettuce has a protective role for the liver against chemicals that may be the cause. It is the ability of the alcoholic extract of the wild lettuce plant to maintain the cell membrane of hepatocytes and thus reduce the leakage of enzymes into the bloodstream and work to curb free radicals and reduce oxidative damage and this was confirmed by (Asif and others, 2020), and does not agree with (Bouimeja). et al., (2019), which demonstrated that the wild lettuce plant, when used as an anti-toxin, causes an increase in the levels of transporter enzymes.

Figure 3: Protective effect of alcoholic extract of L. serriola in the concentration of alanine (ALT)) in male laboratory mice exposed to the carcinogen benzo(a)pyrene.

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