

Article

L. Serriola's Protective Impact on Lung Histology Against Benzo(A)Pyrene-Induced Carcinogenesis in Mice

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Abstract: Natural plants are considered an important factor in human resistance to diseases. This study aimed to evaluate Protective effect of *L. serriole* on histological features of Lungs induced by a carcinogen benzo (a) pyrene. The histological study of the lungs of mice showed that there were significant changes in the normal liver structure in the group of animals that took doses of the carcinogenic substance benzo(a)pyrene, which caused damage to the lung tissue. A disease in the normal structure of the lung tissue in a normal way. The results of the histological diagnosis of the lung showed the presence of hyperemia at the concentration dose of 0.003 mg/kg. When the concentration was given at 0.0015 mg/kg, it was found that there were histological changes, infiltration of inflammatory cells, compared to the control group .The results of changes in the measurements of internal parameters of the lungs in the group that was taken with a carcinogen benzo(a)pyrene only showed a significant increase at the level of probability 0.05 in the average measurements of the internal parameters of the alveoli and bronchi, while the results of the dose of alcoholic extract of wild beer lettuce showed a concentration of 0.006 mg/kg, 0.003 mg/kg, and 0.0015 mg/kg showed a slight increase at a probability level of 0.05 compared to the control group.

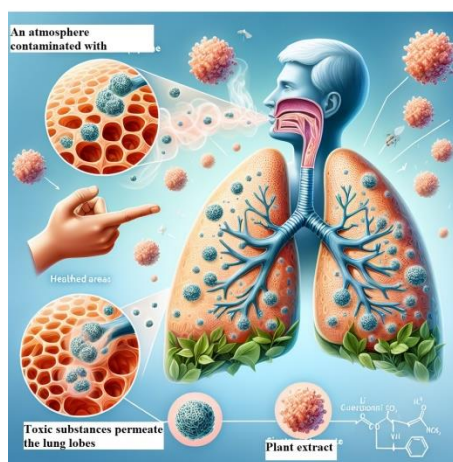
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Keywords: *L. serriole* , benzo (a) pyrene, Lungs tissue, Carcinogens, Histological changes



Graphical abstract of the protective role of *L. Serriole* on benzo (a) pyrene

1. Introduction

Medicinal plants and the effective chemicals extracted from them are considered an important factor in human resistance to diseases. Effective substances were extracted from them, which showed hopeful effects in the treatment of many diseases (El-Esawi et al., 2017; Hameed et al., 2021). Recent research and studies have tended to use the plant in Treatment of many diseases, including cancer diseases, and it has also been used experimentally in inhibiting cancerous cell lines in laboratories, as compounds such as phenolic compounds, flavonoids, and isoflavonoids have been found that have an inhibitory effect on some cancerous cell lines, and recent research has shown that phytochemicals It has anti-cancer activity by targeting a wide range of cell signaling pathways at various levels (Sessa et al., 2000; Mohammed et al., 2020). The wild lettuce plant, *Lactuca serriole* of the Asteraceae family, is rich in active compounds (Koc et al., 2015). These include phenols and flavonoids that are highly effective against cancer (Muniyandi et al., 2019), in addition to their anti-cancer activity , Antioxidant also contains the milk of the latex plant called *Lactucarium* (Ahmed et al., 2016), which is used in medicine because of its many medicinal properties such as its effectiveness as an anti-cancer Anticancer , Diuretic, Emollient, Febrifuge, Hypnotic, Narcotic, Tonic, Galactagogue, Sedative, Anxiety, Neuroses, and joint pain, as indicated by (Janbaz et al., 2013) indicated that the methanolic extract of the aerial parts of the wild lettuce plant *Lactuca serriole* has a significant effect in reducing anxiety and depression as well as symptoms, and the active compounds present in the alcoholic extract of the wild lettuce plant *Lactuca serriole* have many biological activities that include antimicrobials, anti-mitochondrial adhesion, conducted a study on the antagonistic effect of *Lactuca serriole* against scorpion poison *Buthus atlantis*, which showed that the alcoholic extract of wild lettuce plant has a significant protective ability against scorpion poison (Bouimeja et al., 2019; Abdulilah et al., 2019), anti-ulcer (Shukurlu & Goger, 2021). Benzo(a)pyrene is one of the main environmental pollutants resulting from incomplete combustion of organic materials, forest fires, volcanic eruptions, fossil fuels and cracking wood, industrial processes (Singh et al., 2016), where studies confirmed that the association between exposure to polycyclic aromatic hydrocarbons found in Urban air and lung cancer (Majumder et al., 2021), and the study aimed to study the protective effect of the alcoholic extract of wild lettuce plant *Lactuca serriole* in protecting the lung after treating mice with the carcinogen benzo(a)pyrene.

2. Materials and Method

2.1. Preparation of the plant extract

The alcoholic extract 99% was prepared according to the (Hameed *et al.*, 2020), method, by soaking 4g of wild lettuce powder in 100ml of methyl alcohol at a concentration of 99% for 24hrs in a shaking table incubator, filtering using Whatman 1 filter papers. Centrifuged for 10 min. at a speed of 2000 rpm, after that the alcohol was evaporated in the incubator for 48 hrs, then the extract was suspended in 10 ml of distilled water, and three concentrations of 0.006 mg/kg, 0.003 mg/kg, and 0.001 mg/kg were prepared. The carcinogen Benzo(a)pyrene was prepared from Guokang China. The carcinogen was dissolved in vegetable oil according to the method (Alhamdow *et al.*, 2021; Jassim & Hameed, 2020)

2.2. Laboratory animal collection

35 adult male mice were used from the National Center for Drug Control in Baghdad. They were placed in plastic cages for animal breeding in the animal house of the College of Education for Women / Anbar University. The mice were distributed among the cages, with 7 mice in one cage. Mice were subjected to appropriate laboratory conditions in terms of ventilation, temperature and appropriate lighting, while giving them water continuously, and feeding them with standard diet. The protective plant extract was given 30

days before giving the plant the carcinogenic substance. mice were distributed into 5 groups, in each group 7 mice, for a period of 30 days: G1 the negative control group: it was given distilled water only. G2, the positive control group: it was treated with the carcinogen Benzopyrene orally, at a concentration of 0.001 mg/kg, after dissolving it in vegetable oil, using the tool for dosing mice, as the carcinogen was used at a concentration of 0.001 mg/kg, at a rate of one dose per week. G3: It was treated with alcoholic extract of Lactuca plant orally, using the dosing device of gavage mice, as the alcoholic extract of the plant was used at a concentration of 0.006 mg / kg, daily. G4: It was treated with alcoholic extract of *L. serriole* plant orally, using the dosing device of gavage mice, as the alcoholic extract of the plant was used at a concentration of 0.003 mg / kg, on a daily basis. G5:- It was treated with an alcoholic extract of the Lactuca plant orally, using the dosing device of gavage mice, as the alcoholic extract of the plant was used at a concentration of 0.0015 mg/kg, daily.

2.3. Preparation of Histological Sections

The animals were liberated after the expiration of the prescribed treatment period, according to the method of McKenzie (2011), as they were anesthetized with chloroform, then the animals were dissected and the lungs were removed and placed in plastic containers containing formalin at a concentration of 10% for the purpose of biochemical and histological study, They were treated with a physiological solution for the purpose of washing them, then they were cut into small pieces of 1 cm in size and placed in formalin buffer at a concentration of 10% for 48 hrs. After that, the samples are washed with tap water. For a period of 20-30 minutes, in order to get rid of the excess fixative solution, after that, routine methods were applied according to Luna, 1968) in the preparation of slides

2.4. Statistical Analysis

The data were statistically analyzed according to the Complete Randomized Design (CRD) model using the statistical program Genn Stat- Tenth Edition Version and the significant difference of the means were tested using the least significant difference test L.S.D. at the probability level ($p \leq 0.05$) (AL-Rawi and Khalaf ALLah).

3. Results and Discussion

The results of the histological examination of the lung under the light microscope showed that there were histological changes in the lung tissue, according to the treatments, compared with the healthy control group. For a period of 30 days, changes appeared in the normal structure of the lung tissue. These changes were represented by congestion of blood vessels with an increase in the thickness of the lung epithelium, hyperplasia of the lining cells in the bronchioles, a large hyperplasia of smooth cells with infiltration of inflammatory cells, and the destruction of alveoli and pulmonary vesicles, as in (Figure 1A).

The histological diagnosis of the lung were shown in a group of animals that were dosed orally with alcoholic extract of wild lettuce plant *L. serriole* at a concentration of 0.006 mg/kg for 30 days in conjunction with dosing them with the carcinogen Benzo(a)pyrene at a concentration of 0.001 mg/kg in one dose per week for 30 days. days and compared with the control group, the results of the histological diagnosis of the lung tissue were represented by the structure of the lung tissue approaching the normal shape of the lung with congestion of blood vessels as in Figure 1B, While the results of the histological examination of the lung in a group of animals that were dosed orally with an alcoholic extract of wild lettuce plant *L. serriole* at a concentration of 0.003 mg/kg for a period of 30 days and in conjunction with an oral dose of the carcinogen Benzopyrene at a concentration of 0.001 mg/kg at a rate of one dose per week for a period of 30 days and compared with control group, the results showed congestion of blood vessels with infiltration of inflammatory cells as in Figure 1C. When mice were dosed orally with alcoholic extract of *L. serriole* at a concentration of 0.0015 mg/kg for a period of 30 days and simultaneously

with dosed orally with the carcinogen Benzopyrene at a concentration of 0.001 mg/kg at one dose per week for a period of 30 days and compared the results with the control group, the histological diagnosis of the tissues was shown. Lung infiltration of inflammatory cells and congestion of blood vessels as in Figure 1D.

The histological study of the lung in a group of animals that were dosed only with the carcinogenic substance benzo(a)pyrene showed the presence of histological changes in the lung tissue, which is represented by the destruction of the alveoli, pulmonary vesicles, and the congestion of blood vessels, infiltration of inflammatory cells, and these results agreed with (Mohammad et al., 2017), as they indicated that benzo(a)pyrene may cause significant changes in the pulmonary tissue, including an increase in the thickness of the lung epithelium and the destruction consistent with the study (Majumder et al., 2021), which indicated that olive leaf extract, *Olea europaea* L, is rich in phenolic compounds that possess antioxidant and anti-inflammatory properties as well. They protect the lung from cancer caused by benzopyrene and associated with oxidative stress. By preserving the activity of antioxidants, therefore, it was used as a protector against cancer caused by benzopyrene, which caused deformation of the alveoli and an increase in the number of nuclei in the cells of the alveolar wall, in addition to excessive cell proliferation and thus damage to the lung structure in animals that were dosed orally with benzopyrene when compared to animals in the control group.

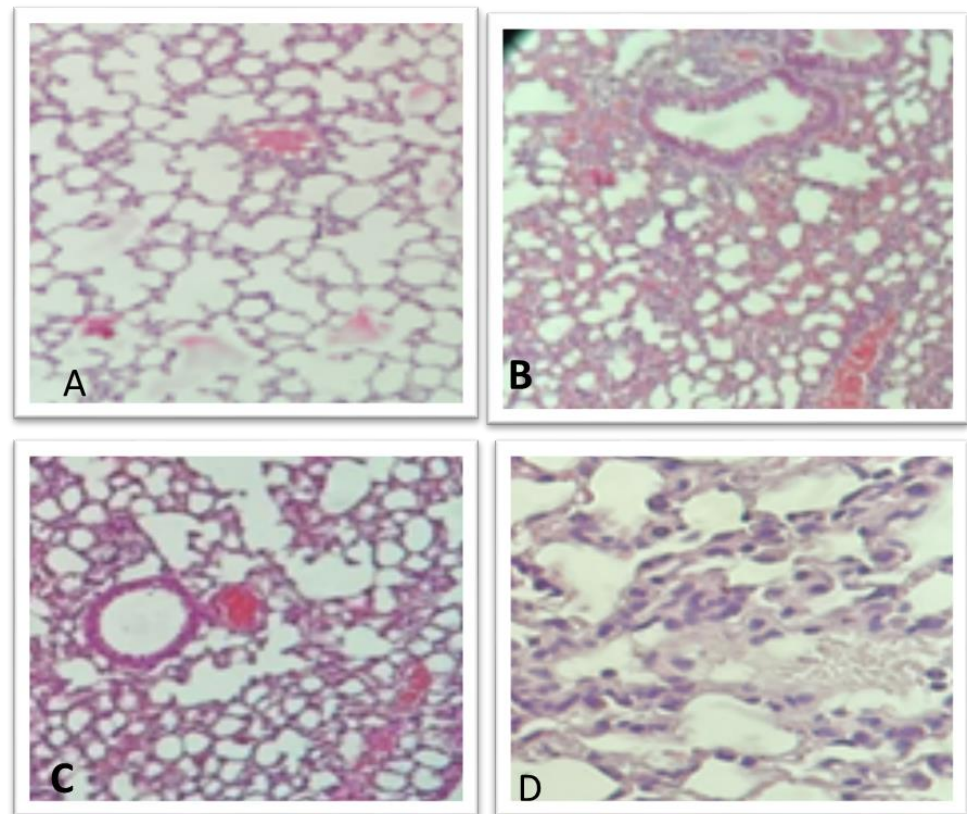


Figure 1. A: Shows no histological changes of lungs in control (H&E) 10x, B: Tissue section of a mouse lung from the group treated with benzopyrene (B(a)p) only showing A_ vascular congestion B_ smooth cell hyperplasia C_ inflammatory cell infiltration 40x, C: Tissues section of lung from the group treated with alcoholic extract of wild lettuce plant *L. serriole* at a concentration of 0.006 mg/kg and benzopyrene B (a)p, showing the histological appearance close to normal with congestion of blood vessels H&E 10x, D: Tissue section in a mouse lung from the group treated with alcoholic extract of wild lettuce plant *L. serriole* at a concentration of 0.0015 mg/kg and benzopyrene B (a)p, showing, Congestion of blood vessels, Infiltration of inflammatory cells 10X.

There are statistically significant differences at the level of $0.05 \geq P$ in the average measurements of the diameters of the internal features of the lung represented by the alveoli and the bronchi. *serriole* at a concentration of 0.0015 mg/kg in conjunction with dosing the carcinogen Benzo(a)pyrene, resulted in a significant increase in the average measurements of the internal parameters of the lung with an average of 0.806 μm for the alveoli and 3.094 μm for the trachea compared with the control group, while when the concentration was dosed at 0.003 mg/kg In conjunction with the administration of the carcinogenic substance Benzo(a)pyrene, it was found that there was a significant increase in the average measurements of the diameters of the internal features of the lung, as the average height, respectively, was 0.767 μm for the pulmonary vesicle and 2.038 μm for the trachea, compared with the control group, when it was dosed at a concentration of 0.006 mg/kg. In conjunction with her dose of the carcinogenic substance Benzo(a)pyrene, it was found that there was a rise in the average measurements of the diameters of the internal features of the kidney, as it reached 0.733 micrometers for the alveolus. pulmonary and 1.767 micrometers for the trachea compared to the negative control group, as well as if the animals were dosed the carcinogen only, while the results of the statistical analysis indicated that there were significant differences with statistical significance when the carcinogen Benzo(a)pyrene was dosed only, as it was found that there was a high Significantly significant in the average of the internal parameters of the lung, as it reached 2.154 μm for the alveoli and 5.110 μm for the bronchi, compared with the control group Figure 2 , that the measurements of the internal parameters of the lung in the groups that were previously dosed with the alcoholic extract of wild lettuce plant *L. serriole*, which showed greater protection against the damage caused by the carcinogen Benzo(a)pyrene in the measurements of the internal parameters of the lung, as the group of animals that showed It was dosed with the alcoholic extract of wild lettuce plant at a concentration of 0.006 mg/kg. The results were very close to the control group more than the other concentrations of the extract. The reason may be the effect of the alcoholic extract of wild lettuce plant *L. serriole* against the inflammation caused by benzo(a)pyrene, and also because of its ability to relieve spasm. It works to remove the spasm of the smooth muscles lining the lung and the trachea. It gave great protection to the internal features of the lung represented by the bronchi. This was confirmed by (Servi & Doğan, 2020), as it proved that the alcoholic extract of the wild lettuce plant has a spasmolytic and bronchodilator effect in addition to its effect in smooth muscle relaxation.

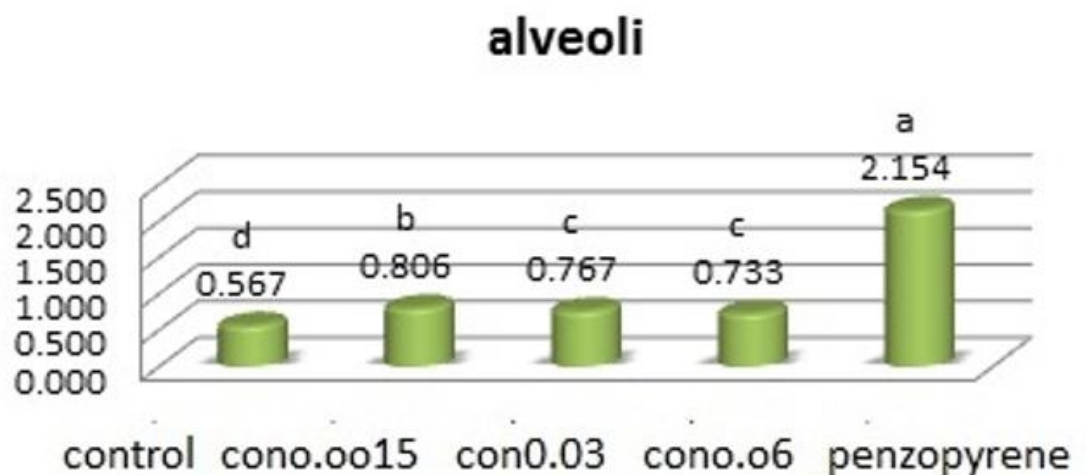


Figure 2. Measurements of alveolar diameters

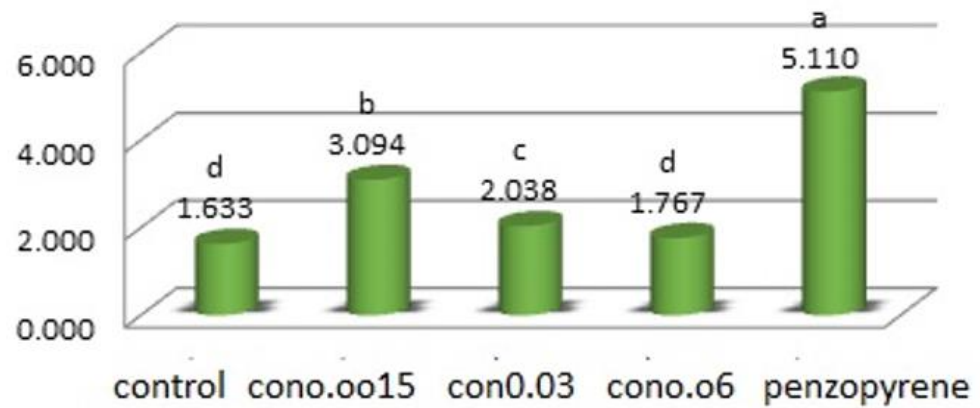


Figure 3. Measurements of tracheal diameters

Figure 3 shows the measurements of the diameters of the internal parameters of the lung for the group that was dosed with the carcinogen Benzo(a)pyrene only. Significant changes occurred in the internal parameters of the lung, including a change in the diameters of the pulmonary vesicle and the bronchi and smooth muscle laceration. The reason may be due to the effects of benzopyrene, which caused with a significant enlargement of both the alveoli and the trachea, it also caused damage to the epithelial layer lining the lung, and thus caused weakness in the vital functions of the lung, and this is consistent with (Rubin, 2021), which proved that low-level exposure to polycyclic aromatic hydrocarbons is closely related to weakness. And decreased vital functions of the lung.

4. Conclusion

The effectiveness of the alcoholic extract in preventing the significant and toxic damage that results from continuous exposure to the toxic substance benzopyrene.

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Conflicts of Interest: The authors declare no conflict of interest.

consistent with the study (Majumder et al., 2021), which indicated that olive leaf extract, *Olea europaea* L, is rich in phenolic compounds that possess antioxidant and anti-inflammatory properties as well. They protect the lung from cancer caused by benzopyrene and associated with oxidative stress. By preserving the activity of antioxidants, therefore, it was used as a protector against cancer caused by benzopyrene, which caused deformation of the alveoli and an increase in the number of nuclei in the cells of the alveolar wall, in addition to excessive cell proliferation and thus damage to the lung structure in animals that were dosed orally with benzopyrene when compared to animals in the control group.

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