

Evaluation the Effect of Alcoholic Extract of *Ruta graveolens* Plant on the Histological Structure of Lungs in the Rabbit

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ABSTRACT

The current study is aimed to find out the effect of *Ruta graveolens* alcohol extract on the histological structure of the lung in rabbits. The study includes the use of 8 rabbits, which were divided into three groups. The first group included a control group that was injected with a physiological saline solution (0.09% NaCl) while the second group is the group of rabbits that were injected with the alcohol extract by a concentration of 250 mg/kg. The third group's rabbits were injected with an extract at a concentration of 500 mg/kg for thirty days for each concentration. The results of the current study showed that rabbits treated with an alcoholic extract of the *Ruta graveolens* plant at a concentration of 250 and 500 mg/kg and for a period of 30 days showed histological changes represented by the occurrence of degeneration and necrosis in the cells of the lining of some bronchi and the separation of some cells from the epithelial layer and the study showed in addition to that, changes in the endothelium lining the blood vessels inside the organ where deformity and congestion in the blood vessel are observed with the appearance of a blood clot inside the blood vessel and the emergence of fibrosis and infiltration of inflammatory cells around the vessel. Vesicular active transport vesicles appeared inside the vessels indicating the high effectiveness of the blood of blood vessels. The study showed that more severe degenerative changes occurred as the concentration of the extract increased on the lung, where a concentration of 500 mg/kg of the extract was observed and for a period of 30 days, increased necrosis and degeneration of the cells in the bronchioles and alveoli with the occurrence of the dissociation of the epithelial layer from the base membrane layer on which it is based and collected in the bronchiole.

Keywords: Alveoli, Bronchiole, lung, Rabbits, *Ruta graveolen* extract.

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INTRODUCTION

Due to the many side effects and negative symptoms as a result of the drug's use and drug's made from chemicals, therefore, attention has been directed to researchers in various fields to search for safer and equally efficient and effective alternatives to these drugs, and these alternatives are plants and medicinal herbs, Among the natural herbs that were used is the *Ruta graveolens*, known as the Rue, a perennial herbaceous plant that belongs to the saponins Rutaceaea family of soaps, which in turn are organized into dicotyledonous plants. The Rutaceae family contains about 160 genera and more than 1,600 species, and there are two main types used in traditional medicine the most important *Ruta graveolens* and *Ruta chalepensis*.¹ The color of the plant is green, bluish to blue up to one meter in

height, and it consists of a group of stems interspersed with side branches and small green leaves topped by a yellow flower that has a strong aromatic scent This flower is completed at the end of the summer Capsule-like.² Ruta leaves are extremely important for treating many diseases due to their important secondary metabolism products such as sterols, furanocoumarins, alkaloids, volatile oils, saponins, flavonoids, amino acids, and. Phenols. The main compounds of the plant are Rutin and Quercetin,³ which inhibit the secretions of the pituitary hormones (follicle-stimulating hormone (FSH), luteinizing hormone (LH) and hypothalamus, which affects the growth and maturation of ovarian follicles and the formation of the yellow body⁴ *Ruta graveolens* has many medicinal uses, including the elimination of negative and positive bacteria for

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dye-cream,⁴ coagulation treatment⁵ eradication of parasites, treatment of high cholesterol,⁶ treatment of cancer. A study of Freyer G et al.⁷ confirmed that a Rutin compound isolated from an orchid plant can be used as an antitumor due to its cytotoxic and anticoagulant activity towards a group of human and animal cancerous cell lines. This study aimed to evaluate the effect of alcoholic extract of *Ruta graveolens* plant on the histological structure of lungs in the rabbit.

METHODOLOGY

The Source of Plants

Ruta graveolens L. was obtained from Baghdad city nurseries, if the leaves of the healthy plants were collected and washed with sterile water in a good way to remove dust and suspended soil, then left to dry at room temperature while stirring constantly. Then it was ground by a hand mill and then used to prepare the hot alcoholic extract of the orchid plant. I used continuous Soxhlet Apparatus using the method mentioned by Akook HA, et al.⁸

Prepare the Potions used in the Study

Effective doses of rabbits were prepared using a half-lethal dose LD₅₀ for extract of *Ruta graveolens* in rabbits if it reaches 5000 mg/kg of body weight,⁹ depending on the half-lethal dose, the concentrations of 250 and 500 mg/kg were chosen, and the rabbits were monitored continuously during the experiments. Each rabbit used in the experiment was injected with the required dose of the extracted subcutaneous plant's alcohol extract every day for 30 days. The concentration of the injected alcoholic extract for rabbits used in the experiment was calculated using the following formula.¹⁰

Whereas : the amount of extract to be injected into rabbits for each of the concentrations in the experiment measured in units (g); D: specific concentration of plant extract; : Weight of rabbits used in the study, which ranged between 800–1200 g.

Animals Used in the Laboratory Experiments and Histological Study

In this study, 8 white rabbits were obtained from the animal house of the Institute for Embryo Research and Infertility Treatment/University of Al-Nahrain, the average weight of which ranged between (800–1200) g and age between 4-10 months. These rabbits were randomly divided into three attorneys, the details of which were as follows: The first group is the control group with 2 rabbits and the second group with 3 rabbits which is the group injected with the extract with a concentration of (250 mg/kg of body weight) daily for 30 days, while the third group with 3 rabbits is A group injected with the alcohol extract of the orchid plant with a concentration of (500 mg/kg body weight) daily for 30 days. At the end of the experiment, the rabbits were anesthetized with chloroform, and then the animals were explained and the lung was removed from its site. After that, the samples were fixed with formalin solution for 24 hours, then washed with tap water and transferred to 70% alcohol for preservation. The histological sections were prepared according to a method (Bancroft and

Gamble). The models were then passed to dehydration, with an ascending chain of ethyl alcohol, then placed with a clearing solution of xylene, embedding with paraffin wax and cut wax molds prepared using a rotary microtome of 7 microns. The extracted glass sections were stained using the Haemotoxylin and Eosin (H&E) stain according to the method used in.¹¹ The colored glass clips of Canada carried a balm, after which the samples were examined and photographed using an optical camera equipped with a digital camera.

RESULTS AND DISCUSSIONS

The results of the current study showed that rabbits treated with an alcoholic extract of the *Ruta graveolens* plant at a concentration of 250 and 500 mg/kg and for 30 days showed clear tissue changes represented by the occurrence of degeneration and necrosis in the cells of the lining of some bronchi and the separation of some cells from the epithelial layer as in Figure 1. This effect may be attributed to the presence of toxic phenolic compounds⁴ proven in the alcoholic extract of *Ruta graveolens*. Also¹² indicated that phenolic compounds have an important action in denaturing proteins and their clotting. These compounds may have receptors on the surface of the cells that make up the walls of bronchioles, alveoli, and alveolar sacs that affected cell proteins, leading to their necrosis and degeneration. The necrotic tissue is considered a foreign body and acts as an irritating irritant action that provokes inflammatory reactions in the neighboring tissues. Also, the dead bronchmoid cells release hydrolysis enzymes on large complex molecules, converting them into smaller molecules, followed by the effect of inflammatory cells in capturing these molecules. This may be one of the causes of rupture in the walls of the alveoli, cysts and bronchioles. In addition, the results of the study showed that the extract of the orchid plant *Ruta graveolens* can affect the endothelium of the

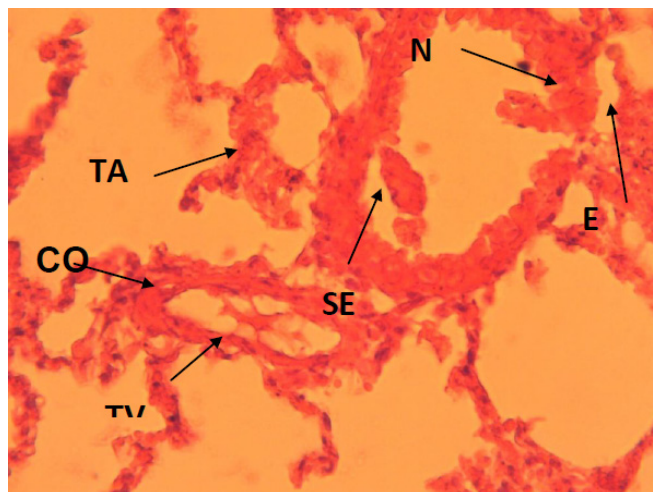


Figure 1: Cross-section of the rabbit lung of the experimental group at a concentration of 250 mg/kg of alcohol extract of *Ruta graveolens* for 30 days, showing the occurrence of degenerative changes in bronchioles. SE separation of some epithelial cells, TA alveolar wall thickness, E edema, N necrosis, co vascular congestion, TV active transport vesicles color (H&E, 40×)

blood vessels inside the organ where deformity and congestion in the blood vessel were observed with the appearance of a blood clot inside the blood vessel and the emergence of fibrosis and infiltration of inflammatory cells around the vessel. Vesicular active transport vesicles appeared inside the vessels, indicating the vascular endothelium's high effectiveness, as in Figures 1 and 2. Perhaps the cause of congestion in the blood vessels is due to the excess blood in the venous system, which led to an increase in blood pressure in the veins and capillaries and this may exert pressure on the neighboring structures, usually accompanied by a decrease in the blood supply, and thus the cells are exposed to malnutrition; consequently, cells are exposed to malnutrition, lack of oxygen and accumulation of excretory substances.¹³ Infiltration of inflammatory cells around blood vessels was a characteristic feature of the

histological sections of lungs. The reason for this is due to the increased effectiveness of the endothelial of the blood vessels in transporting the migratory inflammatory cells from around the body outside the lung into it. Therefore the vacuolation (which contains active transport vesicles inside the vessels) appeared, which explains the presence of the inflammatory cells whose presence indicates their effect. It is important to defend the body and attempt cellular repair. This result is consistent with what many researchers have found.^{14,15} The current study also showed the occurrence of fibrosis in the epithelial layer of the bronchioles, which may be due to the persistence of inflammatory reactions in these cells, which stimulates alveolar cells of the second type to divide and transform into fibroblasts by stimulating materials. This fibroblast begins with the production of collagen fibers. These fibers accumulate without differentiation, thereby causing pulmonary fibrosis, which appeared in some affected tissue regions. This result was consistent with the results of the researchers' study Yao HW, *et al.*,¹⁶ and Al-Hamdany MZ, *et al.*¹⁷ Also, an increase in the wall thickness of some alveoli and alveolar ducts was observed and a break in the alveolar wall Figure 3. The increase in the thickness of the alveoli walls in some areas and the decrease in their alveoli, which may have come as a result of the expansion of alveoli and alveolar sacs, which made this expansion cause pressure on the alveoli forming the edges of the lung, which led to increased compression and made them characterized by an increase in the thickness of their walls and the decrease in their alveoli, and this result is consistent with the study of researchers.¹⁸ The results of the current study showed that the treatment of rabbits with an alcoholic extract of *Ruta graveolens* at a concentration of 500 mg/kg of body weight for 30 days resulted in more severe pathological effects than the previous concentration if it led to increased necrosis and degeneration of cells in bronchioles and alveoli with the occurrence of separation of the epithelial layer on the base

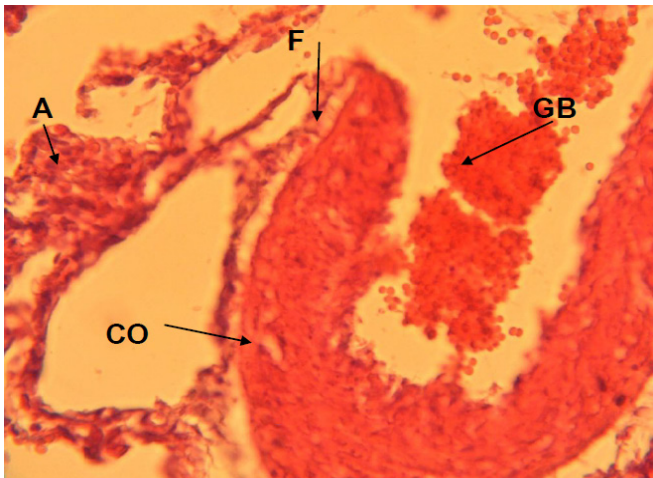


Figure 2: Cross-section of rabbits lung experimental group treated with a concentration of 250 mg/kg of alcohol extract for *Ruta graveolens* for 30 days, showing the occurrence of congestion in the blood vessels of the lung, A infiltration of inflammatory cells, CO blood vessel congestion, GB blood clot., F fibrosis. Color (H&E, 40×)

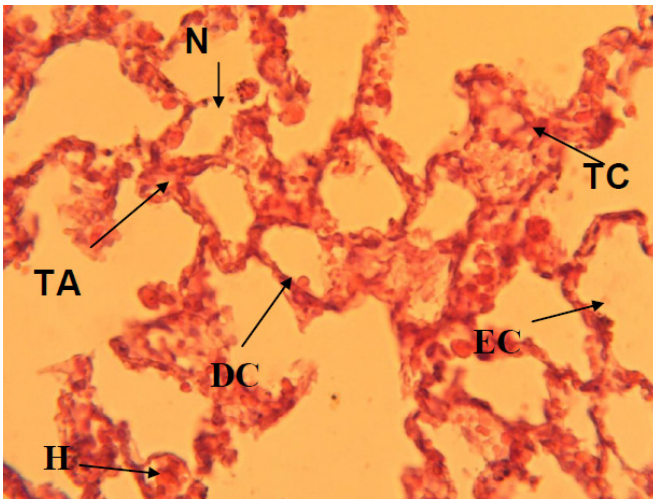


Figure 3: Cross section of rabbits lung experimental group treated with a concentration of 250 mg/kg of alcohol extract for *Ruta graveolens* for 30 days, showing the occurrence of degenerative changes in the lung, TC alveolar sac wall thickness, TA alveolar wall thickness, N necrosis, DC separation of some alveolar cells, EA alveolar expansion, EC alveolar duct expansion, H bleeding into alveolar Color (H&E, 40×)

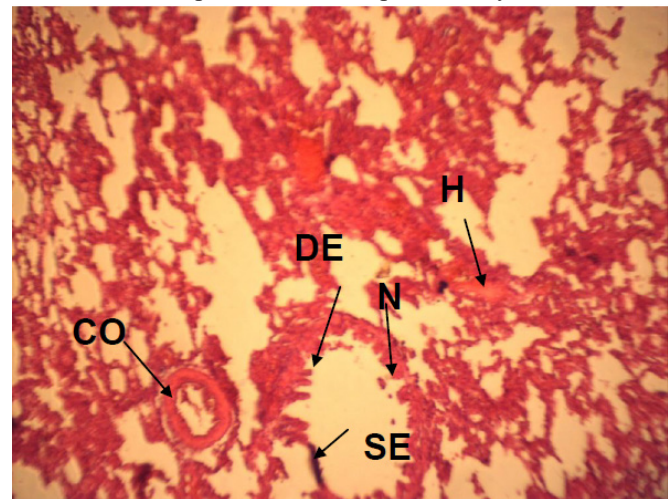


Figure 4: cross section of rabbits lung experimental group treated with a concentration of 500 mg/kg of alcohol extract for *Ruta graveolens* for 30 days, showing the occurrence of degenerative changes of the lung. N, necrosis of bronchioles cells, CO congestion of blood vessels, DE cells destruction epithelial layer, SE separation of some cells of the epithelial layer, intracellular hemorrhage alveoli. Color (H&E, 40×)



Figure 5: Cross section in the rabbit lung of the experimental group with a concentration of 500 mg/kg of alcohol extract for *Ruta graveolens* for 30 days, showing congestion in the blood vessels of the lung. CO Vascular congestion, N necrosis of the bronchioles, DE epithelial cell destruction, TA alveolar wall thickness, A infiltration of inflammatory cells. Color (H&E, 40×)

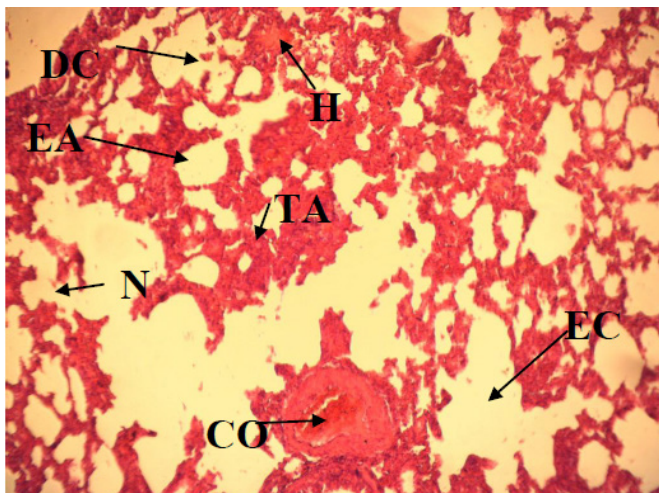


Figure 6: Cross section of the rabbit lung of the experimental group treated with a concentration of 500 mg/kg of alcoholic extract of *Ruta graveolens* for a period of 30 days. It shows the occurrence of degenerative changes in the lung. N, alveolar necrosis, DC separation of some alveoli cells and alveolar cysts, CO vascular congestion, EA alveoli expansion, EC alveolar duct expansion, TA alveolar wall thickness, H intracellular hemorrhage alveoli. Color (H&E, 40×)

membrane layer on which it is based and collected in the lumen of the bronchioles as in Figure 4. Cell necrosis in the lung can be either due to a progressive degenerative action of enzymes within the affected cells or due to a metabolic disturbance or inhibition of the synthesis of DNA and thus hinders the synthesis of proteins necessary for cell growth and maturity.¹⁹ Whereas, the researchers²⁰ indicated that such histological changes can be attributed to the decrease in anti-oxidants in the animal's body due to the chemical present in the extract of *Ruta graveolens*. As it was noticed through Figure 5 the occurrence of bleeding and blood congestion in the blood vessels, and the results showed an expansion in a large number of alveoli

and alveolar sacs and treatment with this concentration led to the occurrence of bleeding in the alveolar wall and a slight thrombus within the alveoli, as well as histological sections showed an increase in the breakdown The alveolar wall of the alveolar ducts, the appearance of edema and vacuolation at different sites of histological sections and the infiltration of inflammatory cells more strongly than in the previous concentration, as in Figure 6. The inflammatory response is believed to be caused by degenerative changes in the lung, as the affected Injuries cell excretes inflammatory factors that lead to the attraction of inflammatory cells as a defensive response to the body to get rid of debris from degenerated cells and this is consistent with the researchers' findings.¹⁸ Among the other manifestations that have been observed is the appearance of a blood clot blocking some capillary vessels in the lungs of treated rabbits. This may be due to the effect of tanning materials on the deposition of proteins, and this may be the cause of deposition of fibrin²¹ blood proteins.

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