# EFFECT OF GLYCYRRHIZIN ON CARRAGEENAN INDUCED LUNG INFLAMATION IN MICE.

***HISTOLOGICAL AND IMMUNOHISTOCHEMICAL STUDY***.

Thesis for partial fulfillment of the master degree of Science of Histology


**By:Mai Tarek Ahmed Kamel**

M.B.B.Ch., Faculty of Medicine, Ain shams University

*Under supervision of*

**Prof. Dr. Naglaa Medhat Abou-Rabia**

Professor of Histology, Faculty of Medicine, Ain shams University

**Prof. Dr. Suzi Sobhy Atalla**

Professor of Histology, Faculty of Medicine, Ain shams University

**Dr. Hala Farouk Fahmy**

Ass. Professor of Histology, Faculty of Medicine, Ain shams University

Histology Department

Faculty of Medicine, Ain shams University


٢٠١١

# ***Acknowledgment***

*First and foremost, thanks* ***Allah*** *the kindest and the most merciful, to whom I relate any success in achieving any work in my life.*

*I find no words by which I can express my deepest gratitude and sincere thanks to* ***Prof. Dr.: Naglaa Medhat Abou-Rabia*** *Professor of Histology, Faculty of medicine , Ain Shams University, for her constructive advices and valuable comments that added much to this work and made this work executed. Any attempt to express my indebtedness to her, will be far from being complete.*

*I am particularly very grateful and appreciative to the keen supervision, great patience and endless help of* ***Prof. Dr.: Souzi Sobhy Atalla,*** *Professor of Histology, Faculty of medicine, Ain Shams University. She has always been helpful in attitude that made the completion of this work much easier than it would otherwise be.*

*My deepest thanks are to **Dr.: Hala Farouk Fahmy** Assistant Professor of Histology, Faculty of medicine, Ain Shams University, for her kind care, honest help and guidance throughout the performance of this work.*

*I want to extend my sincere thanks to all my professors and colleagues in the Histology department, for their valuable advices and continuous support and encouragement.*

## *Dedication*

*Words can not express my thanks, gratefulness, respect and love to my **parents**, my **husband** and all members of my family. Without their help, support, patience and encouragement, I would have never achieved any success.*
*To all of them and to **Laila** & **Youssef,** I dedicate my work.*

*Mai Tarek Ahmed*
*Cairo, 2011*

# List of abbreviations:

| | |
|---|---|
| * Analysis Of Variant: | ANOVA |
| * Before Christ: | BC |
| *Bronchial associated lymphatic tissue: | BALT |
| * Carbon Tetrachloride: | CCl٤ |
| * Carrageenan: | CGN |
| * Cyclooxygenase-٢: | CLAR |
| * Diaminobenzidine tetrahydrochloride: | DAB |
| *Food and agriculture organization: | FAO |
| * Glycyrrhetinic Acid: | GA |
| * Glycyrrhizin: | GZ |
| *High power field: | HPF |
| * Horse Radish Peroxidase: | HRP |
| * Human immunodifficiency virus: | HIV |
| *Intercellular Cell Adhesion Molecule-١: | ICAM-١ |
| * Interleukin: | IL |
| * Kilodalton: | KD |
| * Low Density Lipoprotien: | LDL |
| * Matrix metalloproteinases: | MMP |
| * Myeloperoxidase: | MPO |
| * Nuclear Factor- Kappa B: | NF-κB |

| | |
|---|---|
| * Poly-ADP- ribose: | PDR |
| * Platelet Derived Growth Factor: | PDGF |
| * Polymorphnuclear cells: | PMNs |
| * Probability of significance value: | P value |
| * Reactive Oxygen Species: | ROS |
| * Severe acute respiratory syndrome: | SARS |
| * Signal transducer and activator of transcription ٣: | STAT-٣ |
| * Standard Deviations: | SD |
| *Transforming Growth Factor β: | TGF- β |
| *Tumor Necrosis Factor-α: | TNF-α |
| * World Health Organization: | WHO |
| *Zonula occludens-١: | ZO-١ |

## List of tables:



## List of histograms:

# List of contents

# ABSTRACT

Glycyrrhizin is a saponin compound, a major active constituent of licorice (*Glycyrrhiza glabra*) root and numerous pharmacological effects like anti-inflammatory, anti-viral, anti-tumor and hepatoprotective activities have been attributed to it.

In this study we evaluated the anti-inflammatory activities of glycyrrhizin in mice model of acute inflammation, carrageenan-induced pleurisy. Fifty four male mice were used in this study and were classified into five groups: *GROUP (1)*as control, *GROUP (2)* in which animals received λ-carrageenan *GROUP (3)* in which animals received glycyrrhizin ١٠ mg/kg ,*GROUP (4)* in which animals received glycyrrhizin ٥ minutes before carrageenan and were sacrificed after ٤ hours and *GROUP (5)* in which animals were injected with glycyrrhizin ٥ minutes before carrageenan injection and were sacrificed after ٢٤ hours. We report here that glycyrrhizin (given at ١٠ mg/kg i.p. ٥ min prior to carrageenan) exerts potent anti-inflammatory effects in this model. Injection of carrageenan into the pleural cavity of mice elicited an acute inflammatory response characterized by increase in **١)** thickness of the inter alveolar septa, **٢)** mononuclear cellular infiltration(polymorph nuclear cells and macrophages),**٣)**collagenous fiber content, and a decrease in elastic fiber content and their fragmentation. All these parameters were attenuated by glycyrrhizin as evidenced by different histological stains, immunohistochemistry, semi-thin sections, and morphometric measurements.

Taken together, our results indicate that glycyrrhizin exerts a potent protective anti-inflammatory effect when introduced in a dose of ١٠ mg/kg intraperitonially ٥ min prior to carrageenan which induced acute inflammation in lung.

Keywords: Glycyrrhizin, carrageenan, acute inflammation, lung.

# INTRODUCTION

Licorice (liquorice) or sweetwood is a native herb that is commonly used by the Mediterranean and certain areas of Asia **(Menegazzi, et al., ٢٠٠٨)**. The licorice (liquorice) plant has a long and storied history of use in both Eastern and Western cultures pre-dating the Babylonian and Egyptian empires **(Fenwick et al., ١٩٩٠ and Olukoga and Donaldson, ١٩٩٨).**

The genus name Glycyrrhiza is derived from the ancient Greek word for 'sweet root' (Gr. glykos (sweet) + rhiza (root)), which was later Latinized to liquiritia and eventually to licorice The two principal forms in commerce are licorice root (Liquiriti radix) and the extract (Glycyrrhizae extractum crudum or Succus liquiritiae) which can be used as a sugar substitute, where it has an antioxidant action in food and strengthens food aroma. The sweet taste of licorice is entirely due to glycyrrhizin, which is ٥٠ times sweeter than sugar **(Isbrucker and Burdock, ٢٠٠٦).**

Licorice root is used as a traditional medicine for the treatment of peptic ulcer, hepatitis C, pulmonary and skin diseases. Clinical and experimental studies

suggest that it has an anti-inflammatory, antiviral, antimicrobial ,antioxidative, anticancerous activities, immune-modulatory, hepato-protective and cardio protective effects **(Nassiri and Hosseinzadeh, ٢٠٠٨).**

A large number of components have been isolated from licorice, including flavonoids, isoflavonoids, chalcones and triterpene saponins. From the later glycyrrhizic acid (glycyrrhizin) is considered to be the main biologically active component **(Nassiri and Hosseinzadeh,٢٠٠٨)**.

Glycyrrhizic acid (Glycyrrhizin) stimulates melanogenesis in B١٦ melanoma cells **(Jung, et al., ٢٠٠١)** and has an antithrombotic effect **(Mendes-Silva, et al., ٢٠٠٣)**.In mice it alleviates experimental allergic asthma **(Ram, et al., ٢٠٠٦)** and reduces secondary inflammatory process after spinal cord compression injury**(Genovese, et al., ٢٠٠٨).**

Carrageenan is a high-molecular-weight sulphated poly-saccharide, which is a well established material that is used to assess the contribution of mediators involved in vascular changes associated with acute inflammation. Its

injection into the pleural space leads to pleurisy and acute lung inflammation. **(Corsini, et al., ٢٠٠٥)**.

Very few researches have been done on the anti-inflammatory effect of glycyrrhizin on lung tissues. So, **the aim of this study** is to evaluate carrageenan as a lung-inducing inflammatory agent and to investigate the possible protective and ameliorative role of glycyrrhizin on its structure in mice.

# REVIEW

## I- LICORICE

Licorice plant was cultivated and used in one form or another, long before the birth of Christ. The curative properties of licorice were certainly well known to the Assyrians **(Lucas, ١٩٦٦)** .**Thorwald (١٩٦٦)** reported that Arad-Nana, personal physician to King Asahadon *(680-669 BC)*, prescribed licorice for the relief of rheumatic and other forms of pain. Furthermore, historians believed that Cleopatra might have used licorice with the intent of preserving her beauty. According to **Wheelwright (١٩٧٤)** the earliest written reference to the use of licorice as a medicine was contained in the Codex Hammurabi, dating from *2100 BC.*

Licorice (Glycyrrhiza glabra) is a perennial leguminous plant, widespread in Spain, Italy, Turkey, Israel, Syria, Iran, China and Russia .The plant, having multi-year production- cycle, has blue, violet flowers .Licorice roots are cylindrical in shape having a diameter of ٠,٥–٢,٥ cm and length of ١٥–٢٠ cm. Its fresh root contains about ٢٠٪ of water-soluble extractives, and typically ٣–٥٪ of the root is composed of glycyrrhizin (glycyrrhizic acid; glycyrrhizinate). Glycyrrhizin (GZ) which is the primary active ingredient is present as a mixture of potassium and

calcium salts. Licorice extract also contains ٣-١٦٪ reducing and non-reducing sugars,٣٠٪ starch, plant gums, resins, essential oils, inorganic salts and low levels of nitrogenous constituents such as proteins, amino acids, and nucleic acids **(Fenwick,et al.,١٩٩٠ and Esra bano and Enol bano,٢٠٠٠).**

Licorice was reported to have therapeutic uses for fevers, liver diseases, dyspepsia, gastric ulcers, sore throats, asthma, bronchitis, Addison's disease and rheumatoid arthritis. It had been used as a laxative, anti-tussive and expectorant **(Schulz, et al., ١٩٩٨ and Wang, et al., ٢٠٠٠).**

The bright yellow color of licorice root is provided by flavonoids, particularly liquiritin, isoliquiritin and their corresponding aglycones, which typically comprise ١–١,٥٪ of the water soluble extract. Flavonoids showed anti-Helicobacter pylori activity against a clarithromycin and amoxicillin -resistant strain. Thus flavonoids proved to be useful chemo-preventive agents for peptic ulcer or gastric cancer in Helicobacter pylori-infected individuals **(Toshio, et al., ٢٠٠٢).**

Glycyrrhizin -an active ingredient of licorice- is a saponin compound comprised of a triterpenoid aglycone, glycyrrhetic acid (glycyrrhetinic acid) conjugated to a disaccharide of glucuronic acid. Both glycyrrhizin and glycyrrhetic acid can exist in the ١٨α- and ١٨β-

stereoisomers. Glycyrrhizin represents a mixture of potassium-calcium-magnesium salts of glycyrrhizic acid that varies within a ٢-٢٥ % range. Among the natural saponins, glycyrrhizic acid is a molecule composed of a hydrophilic part, two molecules of glucuronic acid, and a hydrophobic fragment, glycyrrhitic acid**(Fenwick ,et al., ١٩٩٠**).

Glycyrrhetinic acid is the major metabolite of glycyrrhizin that was proved to have a better hepato-protective effect than glycyrrhizin in an in vitro study **(Nose, et al., ١٩٩٤).**Two mechanisms have been suggested for the anti-inflammatory effects of glycyrrhetinic acid. First, glycyrrhetinic acid inhibits glucocorticoid metabolism and potentiates their effects. This potentiation was reported in skin and lung after co-administration of glucocorticoid with glycyrrhetinic acid **(Teelucksingh, et al., ١٩٩٠ and Schleimer, ١٩٩١)**. Since, glycyrrhetinic acid is a potent inhibitor of ١١ hydroxysteroid hydroxygenase, it causes an accumulation of glucocorticoids with anti-inflammatory properties **(Walker and Edwards, ١٩٩١)**.Second, it inhibits classical complement pathway activation and its activity is dependent on its conformation Thus, it was suggested that co-medication of glycyrrhetinic acid with hydrocortisone in the treatment of inflammatory lung disease will be useful **(Kroes, et al., ١٩٩٧).**

Glycyrrhizin was identified as a new thrombin inhibitor as it prolonged plasma recalcification and