

## Treatment Effect of Licorice and Frankincense Eldakr from Pneumonia Induced by Bacterial Liposaccharides in Rats

Safaa M. Faid

Department of Home Economics, Faculty of Specific Education, Ain Shams University, Cairo, Egypt

E mail: [dr\\_safaa2010@yahoo.com](mailto:dr_safaa2010@yahoo.com)

### ABSTRACT

Acute lung injury is a serious illness with a high death rate, resulting in more significant diseases. Thus, this study evaluated the licorice extract for flavonoid compounds and frankincense extract for the essential oil to treat pneumonia in experimental animals. The results found that the major flavonoid compounds in licorice extract were Rutin, Quercetin-3-O-glucoside, Vitexin, and Apigenin-7-O- $\beta$ -glucoside, whilst, Narengin, Quercetin, Luteolin-7-O- $\beta$ -glucoside, and Epicatechin were found in medium amounts. Furthermore, it could be noticed the licorice extract contained antioxidant activity which scavenging free radicals. Meanwhile, Frankincense oil could be identified and the major compounds were  $\alpha$ - Pinene, Limonene,  $\alpha$ - Campholenal, and  $\beta$ -Fenchene whilst, P-Cymene,  $\alpha$ - Thujene, Octanal, and Sabinene were found in medium amounts.

The biological experiment observed that the male rats were divided into five groups; the first group was considered a negative control. The rest were injected with 50 mg/kg of lipopolysaccharide (LPS) to cause pneumonia, after that, re-divided the rat groups. The second group considered as a positive control group, while the 3 and 4 groups were taken orally/day separately 200 ppm /kg BW rat from each licorice and frankincense, and group 5 was taken orally/ day 200ppm from both licorice and frankincense aqueous extract was for four weeks during the experimental period. The results found that group 5 which was taken orally/ day 200ppm from both licorice and frankincense extract give the best results to improve the complete blood picture, inhibition of inflammation and oxidative stress, followed by the groups were taken orally/day separately 200 ppm /kg BW rat /day from each licorice and frankincense, respectively. The result could be recommended to use the licorice and frankincense extract as a new curative agent for treating the pneumonia of rats induced by bacterial liposaccharides.

**Key words:** Licorice, Frankincense, liposaccharides, pneumonia, flavonoids compounds, essential oil

## INTRODUCTION

Acute lung injury (ALI) is the damage of structure and serious oxidation disorders caused by various pathogens, exhibited clinical syndrome, such as respiratory distress and dispersive lung infiltration (**Lv, 2016**). Acute lung injury (ALI) and acute respiratory distress syndrome (ARDS), are described by great hypoxemia, pulmonary edema, and neutrophil accumulation in the lung, which is the prominent source of diseases and mortality in critically disease patients (**Yu et al., 2015**).

Lipopolysaccharide (LPS) is a bacterial endotoxin derived from Gram-negative bacteria. It is also an experiential promoter of acute lung injury (ALI) completely during a week (**Meng et al., 2015**). This process is followed by the development of lung fibrosis (**Moore et al., 2013**). It is activated by stimulating the immune system in a manner similar to severe sepsis by causing systemic inflammation with the production of Reactive Oxygen Species (ROS), which begins to injure the lung tissue by oxidative oxidation of the pulmonary blood vessels. (**Sabarirajan et al., 2010**). This injury ends with severe hypoxemia and inflammatory cell infiltration (**Al-Harbi et al., 2015**). The cell infiltration begins in the capillaries and then in the interstitial tissue and is accompanied by the enlargement of the interstitial fibroblasts. By the time, the defect caused by the tissues and tissue trauma ends with fibrosis (**Matuschak and Lechner, 2010**).

Licorice is an important medicinal substance widely utilized in clinical practice, as it has contained more than 20 triterpenoids and 300 flavonoids. Chalcone, one of the main classes of flavonoids, has a variety of biological activities. Licorice extract has a wide range of pharmacological activities such as anti-inflammatory, antioxidative, anticancer, anti-microorganisms, lowering glucose in the blood, prevention of acute lung injury, heart and liver activities (**Wang et al., 2020 & Editorial, 2020**).

Frankincense has several mechanisms, by inhibiting leukotriene synthesis, cyclooxygenase and lipoxygenase, and oxidative stress, and by regulating immune cells from the innate and acquired immune system. Clinical trials have demonstrated the efficacy of frankincense and its phytochemical against osteoporosis, multiple sclerosis, and asthma. Frankincense showed beneficial influences on brain tumor-related edema that may be a desirable improvement (**Efferth and Oesch, 2020**).

The aim of this study was to study the treatment role of licorice and frankincense Eldakr on lipopolysaccharide-induced lung inflammatory.

## MATERIALS AND METHODS

### Materials

Licorice (*Glycyrrhiza glabra* L.) and frankincense Eldakr (*Boswellia serrata* L.) were purchased from the local market, Cairo, Egypt.

Dried licorice and frankincense were extracted separately in 80% ethanol (100g/ L) by maceration for 72 hours. The extract was filtered using Whatman No1 filter paper. The filtrate was evaporated to concentrate in a rotary evaporator and then stored at 4°C until use (**Darwish et al., 2018**).

Kits for determination of all hematological and antioxidant parameters were purchased from Sigma-Aldrich Corp., MO, for use in analysis. Also, cytokines kits were obtained from enzyme-linked immunosorbent assay (ELISA) kits (R&D systems, Minneapolis, MN, USA). Lipopolysaccharide (LPS) (*Escherichia coli* LPS) purchased from Sigma Aldrich (St. Louis, MO, USA) in phosphate-buffered saline obtained from Sigma Aldrich.

Thirty healthy Sprague–Dawley albino rats weight (200-220g) were purchased from National Organization for Drug and Control Research, Giza, Egypt and they were kept under observation for one week before experiment and fed on the basal diet for growing rats according to **Pell et al., (1992)**.

### Methods:

#### Estimation of antioxidant activity from licorice extract

The total phenolic content in the licorice extract was measured using the method of **Qawasmeh et al., (2012)** with Folin-Ciocalteu reagent. Gallic acid was used as standard (1 mg/ml) and the results were expressed as gallic acid equivalents (GAE mg/g of dry weight). The total flavonoids content was determined by the method of **Eghdami and Sadeghi, (2010)**. The absorbance was measured against a blank solution at 510 nm and the total flavonoids content was expressed in terms of milligrams of quercetin equivalent per gram dry weight (mg QE /g of dry weight).

#### Determination of O<sub>2</sub><sup>•-</sup>, DPPH<sup>•</sup> and FRAP radical solutions

The capability of extracts to neutralize superoxide anion (O<sub>2</sub><sup>•-</sup>) scavenger capacity formed by the reduction of nitroblue tetrazolium (NBT) with NADH mediated by phenazine methosulfate (PMS) under aerobic conditions was conducted according to **Cos et al., (1998)**. The percentage of inhibition I (%) for each radical species was calculated using the following equation:  $I (\%) = 100 \times (A_{\text{blank}} - A_{\text{sample}}) / A_{\text{blank}}$ , where  $A_{\text{blank}}$  was the absorbance of the control reaction and  $A_{\text{sample}}$  was the absorbance of the examined samples, corrected for the value of the blank probe. From the obtained I (%) values, the IC<sub>50</sub> values (which represented the concentrations of the examined extracts that caused 50% neutralization) were determined by linear regression analysis, using Origin software, version 9.0. The DPPH<sup>•</sup> was

prepared and determined according to **Jena *et al.*, (2017)**. The percentage inhibition of the DPPH radical was calculated according to the equation:

$$\text{DPPH scavenging \%} = \left[ \frac{(AC - AT)}{AC} \times 100 \right]$$

Where  $AC$  = Absorbance of the control at  $t = 0$  min

$AT$  = absorbance of the sample +DPPH at  $t = 16$  min

From the obtained I (%) values, the  $IC_{50}$  values (which represented the concentrations of the examined extracts that caused 50% neutralization) were determined by linear regression analysis, using Origin software, version 9.0.

To evaluate the reducing power of extract, the ferric ion reducing antioxidant power (FRAP) determined as described by **Benzie and Strain, (1996)**. Mean values of reducing power were expressed as milligrams of ascorbic acid equivalents (AAE) per gram of dry weight of extract calculated according to the standard calibration curve.

### **Determination of flavonoids fraction from licorice extract**

High-Performance Liquid Chromatography (HPLC) technique was used for separation and estimation of flavonoids compounds in licorice extract was determined according to the method described by **Madrigal-Carballob *et al.*, (2009)**. HPLC instrument (Hewlett Packard series 1100 HP) Column hypersil BDS 5  $\mu\text{m}$  C 18 and Detector UV 254 nm.

### **Essential oil extraction from frankincense:**

Frankincense was hydro-distilled for 3 h in a Clevenger type apparatus according to the European Pharmacopoeia (**Council of Europe, 1997**). The obtained oil was subsequently dried over anhydrous  $\text{Na}_2\text{SO}_4$  and kept at 4 °C until analysis.

### **Gas Chromatography – Mass Spectral Analysis**

The essential oil of frankincense was analyzed by gas chromatography – mass spectral (GC-MS) according to method described in **Ali *et al.*, (2014)**. GC-MS analyses on an Agilent system consisting of a model 6890 Gas Chromatograph, a model 5973 Mass Selective Detector (MSD) and an Agilent Chem Station Data system.

### **Biological experiment**

Experimental male rats were fed on a basal diet for 7 days and randomly divided into five groups six rats of each. The 1<sup>st</sup> main group was fed on a basal diet for another 4 weeks and considered as control negative rats. The rest of the rats were injected with 50 mg/kg of lipopolysaccharide (LPS) by intraperitoneal (IP) injection to induce pneumonia according to **Lee *et al.*, (2019)**, after that, re-divided the rest group. The second group considered as a positive control group was also fed the basal diet only, while the 3 and 4 groups were fed basal diets and taken orally separately 200 ppm /kg bw rat /day from each licorice and frankincense and group 5 was fed basal diets and taken 200ppm from both

licorice and frankincense aqueous extract was together taken orally by stomach tube for four weeks during the experimental period.

At the end of experimental, the blood samples were taken with drawn from the orbital plexus and centrifuged at 3000 rpm to obtain the sera after that, the sera were kept in a deep - freezer at  $-20^{\circ}\text{C}$  until their analysis.

Complete blood of picture as blood hemoglobin (Hb), hematocrite (Ht) and platelets were determined using a whole blood sample and the method described by **Dacie and Lewis, (1984)**, respectively. Red blood cells (RBCs) and white blood cells (WBCs) were measured as recommended by **Riley, (1960)**.

Serum lipid peroxidation was determined calorimetrically as malondialdehyde (MDA) by **Yoshioka et al., (1979)**. Moreover, the activity of the antioxidant enzymes, superoxide dismutase (SOD) was measured in the serum according to the method of **Sairam et al., (2003)**, non-enzyme Glutathione (GSH) was measured in the serum by **Habig et al., (1974)**. Moreover, the activity of the antioxidant enzymes, Catalase (CAT) was measured according to the method of **Aebi, (1995)**.

Serum selected cytokines as interleukin-1 $\alpha$  (IL-1  $\alpha$ ), interleukin-6 (IL-6) were determined according to **Kandir and Keskin, (2016)** and tumor necrotic factor- $\alpha$  (TNF- $\alpha$ ) was determined according to **Millena et al., (2004)**.

### **Statistical analysis**

The obtained data were exposed to the analysis of variance. Duncan's multiple range tests at ( $P \leq 0.05$ ) level was used to compare between means. The analysis was carried out using the ANOVA procedure of Statistical Analysis System (**SAS, 2004**).

## **RESULTS AND DISCUSSION**

### **Antioxidant and its activity in licorice extract**

Polyphenols play a vital role as antioxidant materials which would be the ability to prevent harmful oxidation by scavenging free radicals due to their hydroxyl groups. Therefore, the results In Table (1) revealed that the total phenolic content (TPC) and flavonoids content of licorice aqueous extract had contained 15.59 mg GAE/ g and 10.92 mg QE/g, respectively. Polyphenols act as antioxidants and thus contribute to the relief of a great range of chronic diseases, like cancer, diabetes, skin damage, allergies, atherosclerosis, and viral infections (**Huang and Shen, 2012**).

The results from the same Table showed that the antioxidant capacity (IC<sub>50</sub> value) as determined by DPPH scavenging activity assay and Superoxide anion (O<sub>2</sub><sup>•-</sup>) from licorice extract was found to be good (25.11 and 35.86  $\mu\text{g/ml}$ ), meanwhile, FRAP activity was 55.28 mg/g ascorbic acid equivalents

(AAE). Furthermore, **Sultana *et al.*, (2007a,b)** reported that the great activity of the radical scavenging activity of DPPH referred to as the presence of greater levels of total phenols and flavonoids for the reason that they play the main role as a proton-donating and can act as free radical inhibitors, and may act as an antioxidant.

Phenolic compounds like flavonoids, alkaloids, tannins, and saponins present in licorice extract had contained natural antioxidant activity (**Fangliang *et al.*, 2020**). It could be concluded that the licorice aqueous extract can be utilized as a high source of natural antioxidants in scavenging oxidative harmful in the human body.

**Table (1): Antioxidant and its activity in licorice extract**

Antioxidant and its activity	Licorice extract
*Total phenolic content (TPC)	15.59±0.51
**Total flavonoids compounds	10.92±0.43
***DPPH scavenging activity (IC <sub>50</sub> )	25.11±0.62
****Superoxide anion (O <sub>2</sub> •-)	35.86±0.81
FRAP****	55.28±0.94

Data represented in means of duplicates ± standard deviation.

\*Total phenolic was expressed as gallic acid equivalents (GAE) mg/ g sample

\*\* expressed as quercetin equivalents (QE) mg/ g \*\*\*Inhibitory concentration

at which 50% of DPPH radical is scavenged IC<sub>50</sub> (μ/ml)\*\*\*\* Inhibitory

concentration at which Inhibitory concentration at which 50% of (O<sub>2</sub>•-) radical

is scavenged IC<sub>50</sub> (μ/ml):. \*\*\*\*\* FRAP expressed as milligrams of ascorbic

acid equivalents (AAE) per gram of dry weight

### **Fractionation flavonoids fraction from licorice extract**

The flavonoids compounds from licorice extract were fractionated using HPLC and the results are reported in Table (2). From the results it could be found that thirty compounds from licorice extract could be identified to the major compound was found as Rutin, Quercetin-3-O-glucoside, Vitexin, and Apigenin-7-O-β-glucoside (32.0, 29.0, 28.0, and 25.0 mg/100g), whilst, the compounds, Naringin, Quercetin, Luteolin-7-O-β-glucoside, and Epicatechin had contained medium amounts 18.0, 17.0, 15.0 and 13.0 mg/100g, respectively. Moreover, Kaempferol-7-O-glucoside, Kampferol, Apegnin, Luteolin, and Myricetin as manor compounds had concentrated 4.36, 3.40, 2.41, 9.0, 7.0, and 5.0 mg/100g, respectively. These results are in agreement with **Siracusa *et al.*, (2011)** who found the most well-known flavonoids like rutin, quercetin, lutein, naringenin, kaempferol-7-O-glucoside in licorice has been discovered in previous reports but not quantified. These compounds were found as an antioxidant, anti-inflammatory, and anticancer activity.

**Table (2): Flavonoids content (mg/100g) from licorice extract**

Flavonoid compounds	Flavonoids (mg/100g)
Apegnin	5.00
Narengin	18.00
Luteolin	6.00
Kampferol	7.00
Rutin	32.00
Quercetin	17.00
Myricetin	4.00
Epicatechin	13.00
Vitexin	28.0
Apigenin-7-O- $\beta$ -glucoside	25.00
Luteolin-7-O- $\beta$ -glucoside	15.00
Kaempferol-7-O-glucoside	9.00
Quercetin-3-O-glucoside	29.00

### Quantification and identification of frankincense essential oil

From the results in Table (3), it could be noticed that twelve compounds of frankincense oil could be identified and the major four compounds were found as  $\alpha$ - Pinene, Limonene,  $\alpha$ - Campholenal, and  $\beta$ -Fenchene (15.0, 12.0, 11.0, and 10.0 mg/100g) beside four medium compounds, P-Cymene,  $\alpha$ -Thujene, Octanal and Sabinene were contained amounts 8.0, 7.0, 6.0 and 5.0 mg/100g, respectively. Meanwhile, Ritenol acetate, trans-Pinocarveol, Terpinene and Tricyclene as four minor compounds had concentrated 4.0, 3.0, 2.0, and 1.0 mg/100g, respectively. These results are in agreement with **Kohoude *et al.* (2017)** who observed that the essential oils of *Boswellia dalili* have a large proportion of  $\alpha$ -Pinene, although other main components in those oils like  $\delta$ -3-carene,  $\alpha$ -terpinene, and p-cymene were shown in lower amounts in the oleoresin essential oil. *Boswellia dalzielii* had contained a high amount of monoterpenes, particularly  $\alpha$ -pinene.  $\alpha$ -pinene dominates the essential oils of *Boswellia species*, in addition, lower amounts of  $\alpha$ -thujene, limonene, myrcene, sabinene, and P-cymene (**DeCarlo *et al.*, 2018**).

**Table (3): Identification of essential oils from frankincense**

Identification	Essential Oil (mg/100g)
$\alpha$ - Thujene	7.00
$\alpha$ - Pinene	15.00
$\beta$ -Fenchene	10.00
P-Cymene	8.00
Limonene	12.00
Sabinene	5.00
$\alpha$ - Campholenal	11.00
trans-Pinocarveol	3.00
Terpinene	2.00
Tricyclene	1.00
Octanal	6.00
Ritenol acetate	4.00

### Effect of Licorice and Frankincense and both on hematological in pneumonia of rats induced by bacterial liposaccharides

Hematological analysis was determined in different rat groups treated with licorice, frankincense, and both compared with the control rat health group and the results are reported in Table (4). From the results it could be observed that the lowest hemoglobin (Hb), hematocrit value (Ht), red blood cells (RBCs), and white blood cells (WBCs) in rats control positive (group 2) fed on basal diet were 6.8g/dl, 20.1%, 2.11  $10^6/\mu\text{l}$ , and 7.91  $10^3/\mu\text{l}$ , respectively, compared with the highest healthy rats group was 13.8g/dl, 45.1%, 4.48  $10^6/\mu\text{l}$ , 12.47  $10^3/\mu\text{l}$ , respectively.

The hematological analysis in rats group 5 was received daily basal diet and taken orally/day licorice 100 ppm plus frankincense 100ppm the highest was 12.5g/dl, 44.05%, 4.25  $10^6/\mu\text{l}$ , and 11.87  $10^3/\mu\text{l}$ , followed by the rats' group fed on basal diet and taken orally/ day 200 ppm from licorice extract was 11.1 g/dl, 40.2%, 3.85  $10^6/\mu\text{l}$ , and 10.76  $10^3/\mu\text{l}$ , respectively. Moreover, the rat group 4 has daily received basal diet plus Frankincense 200ppm extract was given orally was 9.9 g/dl, 35.1%, 3.15  $10^6/\mu\text{l}$ , and 9.35  $10^3/\mu\text{l}$ , respectively.

The greatest considerable in erythrocytes and hematocrit counts after oral management of an extract from licorice and frankincense and both may be due to the extract had contained phytochemicals that carried out the secretion of erythropoietin in the stem cells of healthy rats. Erythropoietin is a glycoprotein hormone that encourages stem cells in the bone marrow to produce red blood cells (Ohlsson and Aher, 2009). Erythropoietin impacts the blood's ability to carry oxygen and the amount of oxygen delivered to tissues for the reason, that red blood cells and hemoglobin are important in transporting respiratory gases (Oyediji and Bolarinwa, 2013).

**Table (4): Influence of Licorice and Frankincense and both on some hematological parameters in pneumonia of rats induced by bacterial liposaccharides**

Groups	Hb (g/dl)	Ht (%)	RBCs ( $10^6/\mu\text{L}$ )	WBCs ( $10^3/\mu\text{L}$ )
Control negative (G1)	13.8±0.43 <sup>a</sup>	45.1±0.4 <sup>a</sup>	4.48±1.8 <sup>a</sup>	12.47±0.03 <sup>a</sup>
Control positive (G2)	6.8±0.14 <sup>d</sup>	20.1±0.17 <sup>d</sup>	2.11±0.25 <sup>c</sup>	7.91±0.03 <sup>d</sup>
Licorice 200 ppm (G3)	11.1±0.86 <sup>b</sup>	40.2±1.15 <sup>b</sup>	3.85±0.43 <sup>b</sup>	10.76±0.05 <sup>b</sup>
Frankincense 200 ppm (G4)	9.9±0.78 <sup>c</sup>	35.1±0.51 <sup>c</sup>	3.15±0.57 <sup>b</sup>	9.35±0.06 <sup>c</sup>
Licorice 100 ppm + Frankincense 100ppm (G5)	12.5±0.73 <sup>a</sup>	44.5±2.49 <sup>a</sup>	4.25±0.76 <sup>a</sup>	11.87±0.04 <sup>a</sup>

All values are mean ± standard deviation (n = 3). Values in the same column with different letters are significant at  $P \leq 0.05$



### Effect of Licorice and Frankincense and both on antioxidants in pneumonia of rats induced by bacterial liposaccharides

The antioxidant enzymes and the malondialdehyde (MDA) were determined in different rats groups treated with Licorice and Frankincense and both and compared with the control rat health group and the results are reported in Table (3). The results showed that the rats control positive was the lowest in all parameters by 10.65, 6.28, and 4.08 U/L and also, MDA was the highest by 315.27 nmol/ml, respectively, and the rats control negative group was the highest in all antioxidant enzymes (20.36, 14.38 and 7.53 U/L) and also, the lipid peroxidation as malondialdehyde (MDA) was the lowest by 180.68nmol/ml, respectively.

The rats' groups were taken orally 200 ppm of an extract from licorice and frankincense and both were increased the antioxidant defense, that is, enzymes glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT), increased significantly, whereas, a significant decrease in lipid peroxidation as malondialdehyde (MDA). Moreover, the results reported that the licorice had contained high amounts of flavonoids and phenolic acid, meanwhile, frankincense was the highest in essential oil. When the rats fed on basal diet and taken orally/ day 100ppm from licorice plus 100ppm from frankincense give the best results followed by 200ppm from Licorice and 200ppm from frankincense in rat inducted with acute lung inflammation. These results confirm with **Chen *et al.*, (2017)** which reported that polyphenols elevate the antioxidant enzymes containing superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) proteins which related to antioxidant mechanisms. Pulmonary damage caused by a great in lipid peroxidation may be due to free oxygen radicals and lowering in antioxidant factors. Previous research found that elevated the amount of malondialdehyde (MDA) in animal lungs, decreased levels of both enzymatic and non-enzymatic antioxidants, and caused severe DNA damage (**Afsar *et al.*, 2018**).

**Table (5): Influence of Licorice and Frankincense and both on antioxidant and oxidative stress in pneumonia of rats induced by bacterial liposaccharides**

Groups	GSH (U/L)	SOD (U/L)	CAT (U/L)	MDA (nmol/ml)
Control negative (G1)	20.36 <sup>a</sup> ±1.73	14.38 <sup>a</sup> ±0.77	7.53 <sup>a</sup> ±0.08	180.68 <sup>d</sup> ± 4.53
Control positive (G2)	10.65 <sup>d</sup> ±0.95	6.28 <sup>d</sup> ±0.47	4.08 <sup>d</sup> ±0.07	315.27 <sup>a</sup> ± 7.25
Licorice 200 ppm (G3)	17.59 <sup>b</sup> ±1.05	12.56 <sup>b</sup> ±1.04	6.39 <sup>b</sup> ±0.05	200.53 <sup>c</sup> ± 2.19
Frankincense 200 ppm (G4)	14.67 <sup>c</sup> ±0.98	10.39 <sup>c</sup> ±0.85	5.48 <sup>c</sup> ±0.01	220.75 <sup>b</sup> ± 2.38
Licorice 100 ppm + Frankincense 100 ppm (G5)	19.72 <sup>a</sup> ±1.28	13.94 <sup>a</sup> ±0.91	7.11 <sup>a</sup> ±0.03	185.28 <sup>d</sup> ±1.58

All values are mean ± standard deviation (n = 3). Values in the same column with different letters are significant at  $P \leq 0.05$

### **Effect of Licorice and Frankincense and both on the levels of cytokines in pneumonia of rats induced by bacterial liposaccharides**

Table (6) showed the effect of licorice and frankincense and both on the cytokines TNF- $\alpha$ , IL-1 $\alpha$ , and IL-6 in pneumonia of rats induced by bacterial liposaccharides. From the results, it could be observed that the TNF- $\alpha$ , IL-1 $\alpha$ , and IL-6 were the highest in control positive group rats induced with pneumonia by 200.15, 70.35, and 75.39 pg/mL, respectively. It is known that inflammation and oxidative stress play a vital influence in the pathogenesis of acute pneumonia. Different inflammatory mediators, like single-cell chemical protein 1 (MCP-1), tumor necrosis factor - $\alpha$  (TNF- $\alpha$ ), and interleukin 16 (IL-16), have been implicated in Lipopolysaccharide (LPS)-induced acute lung injury (**Jing and Chunhua, 2015**).

The control healthy rats fed on basal diet were the lowest in the level cytokines by 75.38, 10.25, and 15.18 pg/ml, respectively. In addition, the effect of licorice and frankincense and both on the pneumonia of rats, the results reported that the rat group taken orally/ day with licorice 100ppm extract plus 100ppm frankincense extract was the lowest and nearly control healthy rats group by 59.61, 78.28, and 73.33%, respectively followed by rats group taken orally/ day with Licorice 200ppm extract was 39.78, 63.91, and 60.21%, respectively. Meanwhile, the rats group taken orally/ day with 200ppm Frankincense extract was decreased by 25.00, 49.50, and 45.70%, respectively. These decreases in treatment rat groups may be able to be the licorice is widely utilized in folk medicine to treat many ailments such as infection and inflammation. The Licorice has been shown to have performed anti-inflammatory activity. In acute lung injury models, it is inhibition NF- $\kappa$ B activation, phosphorylation, and extracellular regulated protein kinesis (ERK) may be due to the Licorice contains Coumadin's and flavonoids compounds all these compounds are responsible for the pharmacological activity of licorice (**Salim et al., 2018**). Moreover, Frankincense is an aromatic gum resin that has been in circulation for thousands of years in order to treat infections, and today it is frequently distilled as an essential oil consisting largely of major compounds like  $\alpha$ -pinene,  $\alpha$ -thujene, limonene, sabinene,  $\alpha$ - Campholenal and p-cymene. (**Johnson et al., 2019**).

**Table (6): Influence of Licorice and Frankincense and both cytokines in pneumonia of rats induced by bacterial liposaccharides**

Groups	TNF- $\alpha$	IL-1 $\alpha$	IL-6
	pg/mL		
Control negative (G1)	75.38 <sup>d</sup> $\pm$ 1.84	10.25 <sup>d</sup> $\pm$ 0.91	15.18 <sup>d</sup> $\pm$ 0.91
Control positive (G2)	200.15 <sup>a</sup> $\pm$ 3.27	70.35 <sup>a</sup> $\pm$ 1.28	75.39 <sup>a</sup> $\pm$ 1.63
Licorice 200 ppm (G3)	120.53 <sup>c</sup> $\pm$ 1.97	25.19 <sup>c</sup> $\pm$ 0.28	30.0 <sup>c</sup> $\pm$ 0.41
Frankincense 200 ppm (G4)	150.11 <sup>b</sup> $\pm$ 1.84	35.53 <sup>b</sup> $\pm$ 0.31	40.94 <sup>b</sup> $\pm$ 0.52
Licorice 100 ppm + Frankincense 100ppm (G5)	80.24 <sup>d</sup> $\pm$ 1.86	15.28 <sup>d</sup> $\pm$ 0.94	20.11 <sup>d</sup> $\pm$ 0.81

All values are mean  $\pm$  standard deviation (n = 3). Values in the same column with different letters are significant at  $P \leq 0.05$

## CONCLUSION

From the obviously results it could be concluded that the licorice extract had contained high amounts of flavonoids and utilized in folk medicine to treat many ailments. Meanwhile, Frankincense extract had consisted of the major compounds from the essential oil that was used for medicine in inflammation. Thus, could be used licorice and frankincense extract may treat the pneumonia of rats induced by bacterial liposaccharides, and also, inhibition of inflammation and oxidative stress, therefore, which could a new curative agent for the protection from acute pneumonia.

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## الملخص العربي

### العرقسوس واللبن الدكر كمستخلصات للعلاج من الالتهاب الرئوي الناجم عن الليبوسكريد البكتيري في فئران التجارب

يعتبر الالتهاب الرئوي هو مرض خطير مما يؤدي الى ارتفاع معدل الوفيات. لذلك اعدت هذه الدراسة لتقييم مستخلص العرقسوس لمركبات الفلافونويد ومستخلص اللبن للزيوت العطرية وذلك لمحاولة تقديم علاج من المستخلصات الطبيعية لعلاج الالتهاب الرئوي ، وقد اوضحت النتائج أن مركبات الفلافونويد الرئيسية في مستخلص العرقسوس هي Rutin, Quercetin-3-O-glucoside, Vitexin, and Apigenin-7-O-β-glucoside, بينما احتوت المركبات ، Naringin ، Quercetin ، Luteolin-7-O-β-glucoside ، و Epicatechin بكميات متوسطة. علاوة على ذلك ، يمكن ملاحظة أن مستخلص العرقسوس يحتوي على نشاط مضاد للأكسدة يقضي على الجذور الحرة. وفي الوقت نفسه ، يمكن التعرف على المركبات الرئيسية في زيت اللبن مثل α- Pinene, Limonene, α- Campholenal, and β-Fenchene بجانب المركبات المتوسطة كانت P- Cymene و α- Thujene و Octanal و Sabinene. ومن خلال التجربة البيولوجية للفئران والتي قسم فيها ٣٠ من ذكور فئران التجارب إلى خمس مجموعات. المجموعة الأولى كانت تعتبر المجموعة الضابطة السالبة حيث تغذت على الوجبة الاساسية دون أي اضافات ثم تم حقن الفئران المتبقية بحقن ٥٠ مجم / كجم من الليبوسكريد البكتيري (LPS) لكي تسبب لهم الالتهاب الرئوي ، وبعد ذلك تم إعادة تقسيم مجموعات الجرذان المصابه حيث اعتبرت المجموعة الثانية مجموعة ضابطة إيجابية ، بينما أخذت المجموعة الثالثة والرابعة عن طريق الفم / يوميا بشكل منفصل ٢٠٠ جزء في المليون / كجم من وزن جسم الفأر / يوميا من كل من العرقسوس واللبن ، والمجموعة الخامسة تناولت ٢٠٠ جزء في المليون عن طريق الفم / اليوم من كل من العرقسوس واللبن معاً لمدة أربعة أسابيع خلال فترة التجربة. اوضحت النتائج أن المجموعة الخامسة التي أخذت عن طريق الفم ٢٠٠ جزء في المليون / يوم من كل من خلاصة العرقسوس واللبن تعطي أفضل النتائج فحدث تحسين لصورة الدم الكاملة ، وتنشيط الالتهابات والإجهاد التأكسدي ، تليها المجموعات التي تم تناولها عن طريق الفم ٢٠٠ جزء في المليون / كجم. وزن الجسم للفئران / يوميا من كل من العرقسوس واللبن بشكل منفصل على التوالي. ومما سبق ، يمكن التوصية باستخدام مستخلص العرقسوس واللبن التي تم تجربتها لعلاج مجموعات الفئران التي تعاني من الالتهاب الرئوي الناجم عن الليبوسكريد البكتيري (LPS) ، والتي يمكن أن تكون عاملاً علاجياً جديداً للمساهمة في علاج الالتهاب الرئوي.

**الكلمات المفتاحية:** العرقسوس- لبن الدكر- الليبوسكريد- المركبات الفينولية- الزيوت الاساسية