Histological and ultrastructural studies on the effect of Costus Plant and Amphotericin B on male lung rats infected by *Aspergillus niger*

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Abstract: This work amid to study the use of costus plant in the treatment of pulmonary infection caused by *Aspergillus niger* instead of the chemical drug (Amphotericin B), The experiments were conducted using 90 white male rats that were divided into the following groups : Group one consisted of the control 30 rats which orally administrated with distilled water. Group two comprised 10 rats treated with the fungus suspension. (0.4mg/ kg b.wt.) Group three comprised 40 rats treated with costus plant extract and divided into four subgroups. Group four comprised 20 rats treated with Amphotericin B (0.2mg/ kg) which was divided into two subgroups, then sacrificed and dissected. Then biopsies were taken from the lunges of the various groups of rats and placed in various fixatives in order to conduct the histological and ultrastructural studies. It could be observed from examination of the histological and ultrastructural sections of the lungs of the rats infected with the fungus, the appearance of numerous lymph inflammations especially around the bronchioles. In addition, some reduced with degenerative walls, In case of rats treated with costus extract, the lung tissues appeared normal. Also the rats infected with fungus then treated with costus, the interstitial tissues nearly restored its normal shape and appeared free from cytoplasmic degeneration. On the other sides the histological and ultrastructural sections of rats treated with Amphtericin B after fungus infection showed deformed lung tissues.

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1. Introduction

I-Aspergillus niger:

Alexopoulos and Mims,(1979) The Family : Eurotinceae, is considered from the important families because it comprise famous genera with of the major factions great economic importance (Alexopoulos and Mims,1979). This Family is called also Aspergillaceae because the conidial fungus were obvious which preferred in studies than the complete phases. *Aspergillus niger* one of the most famous species of the genus *Aspergillus*.

Species belonging to this genus are from the most non living medium prevalent in nature, where fungus spores present in the soil,air and grow on any one of the most causes of pollution in the laboratories. Aflatoxil B was discovered in 1962.

1-Aflatoxil B1:

The most of species are harmful and cause diseases for Humans and animals which known as *Aspergillosis*. Furthermore, Hoshino *et al.* (1984) indicated that with fungal infection *A.niger* cause Allergic bronchopulnonary *Aspergillosis*.

According to Bennett (1980), the role played by *Aspergillus* in lung diseases is not clear, where as the Fungal hyphae bronchitis, in addition, the effects and the sensitivity to anti fungals that can. cause serious damage to bronchial constriction, and that the fungal invasion of lung tissue confined entirely in patients suffered from immune deficiency.

The infection with *Asperigillus* is characterized by the presence of inflammations,of the skin, external ear, sinus, eye socket, eye, lungs, pleura, chest cavity, bones, brain and meninges, heart valves, rarely throat, vagina and uterus Mashni (1998).

Abdel Hameed (2000) concluded that the spore extracts of some species of *Asperigillus* have carcinogenic effect on the young ducks. Johnson *et al*., (1998) indicated that the development evolution of SARS arises from abnormal immune system unable to resist infections, causing an inability to blood oxygenation, leading to lethargy and shortness of breath associated with excessive mucus secretions in the airways leads to coughing breathing and difficulties and an increase in the number of white blood cells, neutrophils and monocytes.

The diagnosis for this disease indicated inflammation of the air bags and narrow bronchi and pulmonary Odema and an increase in the number of pulmonary acidic blood cells (Davidson, 2006)

II-Costus:

Costus means in Arabic Oud, so it is possible to say marine costus or marine Oud, because Arabs brings it from the sea.

There are two types of Costus, the first is marine or white or sweet, and the second type is an Indian or

black or bitter, and the Indian is more hotter than the marine type.

The Oud is taken from Costus germling whith 1.5 mm length which has leaves, stem and root, and present in India.

The root peels are the active therapeutic used parts which are white or black (Alamuel, 2006).

Scientific classification of plants Costus:

Kingdom : plant
Division : Spermatophyta
Sub.D: Angiospermae
Class : Monocotyledoneae
Order : Scitamineae
Family :Zingiberaceae

Lawrence (1969) has pointed that the family Zingiberaceae divided into two sub families Zingiberadeae and Costusedeae.

The second one comprised four species from which the genus Costus.

The benefits and medical uses of Costus plant :

Al-Tirmidhi narrated from Zayd ibn Arqam that the prophet peace be upon him said: (Treat pleurisy installment by marine Costus and oil).

In the novel of the Albokhari from Umm Qays bent Muhsin said: I heard the prophet peace be upon him say: (you must use the Indian Oud which has seven cures: Istatt its virginity and the evidence of pleurisy).

Also, prophet peace be upon him said(which has seven cures) Bukhaari said: Rawi said: "I heard Al-zahrni say: he showed us two not five Hajar said: signed as well as the shortcut to talk about two of the seven is either shortened by the narrator or talk about two for its existence only. And he (the evidence of pleurisy) means Isagah in a naughty mouth, a warning as to the method of watering when the remedy for the patient to sit Aeetmkn believe eating raises his hand when he had severe pain arch is watered rights in a naughty mouth.

Baghdadi said the conciliator (and collected peace be upon him straight between the cupping and the secret to a nice, if painted by a scalpel cupping with no lag in the skin effect Almcharit This is an oddity of medicine, these effects if it grew in the skin may fancy that they saw the flash and vitilligo or dislike where these effects with the cupping aware of what he believes it is.

The Costus may make it the prophet be upon him represent what many take any medicine by the benefits of semi-paralysis and move the benefit Beh, a snake venom antidote, and Achtmamh dispelled by the cold, fat and useless back ache.

Vool and Maleeva (1952) stated that there are many kinds of Costus, they emphasized the medical benefits of three types: *Costs arabicus* which is common in South-East Asia and cultivated in India and Indonesia and is used therapeutically in the chest diseases cough and asthma.

Costu afrri which is common in tropical Africa, the powder of dried stems used for cough treatment whereas, the dye prepared from roots used in a pharmaceutical formulation for sleeping sickness, leaves are boiled and applied topically to treat Alrthyp, and the boiled roots used as local cure to heal skin ulcers. *Costus spicatus* is wide spread in Colombia and tropical America, especially in Peru, Guyana and used medically to treat chronic bronchitis and enteric fever and typhus.

Dutt ***et al.*** (1960) and Sastry & Dutta (1961) confirmed the effectiveness of Costus plant in the treatment of chronic bronchitis inflammation and asthma.

Also, Cruz (1965) found that the injection with derial parts of Costus was effective in the treatment cold and sore throats, dysentery and diarrhea.

The results obtained by Whistler ***et al.*** (1976) indicated the polysaccharides present in Costus and the use of its roots in traditional medicine in Brazil improve the phagocytosis and provide vascular protection in the reticulo-endothelial system of the blood vessel.

Otrero *et al.*(2000) proved that 13 of the 74 extracts of Costus,used by the premium traditional healers to snake bites in the north-western Colombia were effective against the lethal effect of the poison Bothropsatrox.

Pandey *et al.*(2007) isolated many biologically active compounds from Saussurea Costus which were costunolide, dehydrocostus lactone and cynaropicrin. So it is appropriate to develop its use as medicine.Vijayalakshmi &Sarada (2008) stated that the polyphenols content of extracts of Costus than in leaves was higher in the roots and stem cortex. Thus, they proved the effectiveness of these parts as alternative for chemical antioxidants.

III-Amphotericin-B:

Amphotericin-B was discovered in (1956) and isolated from the spore of *Streptomyces nodosus* and the radial anaerobic fungus isolated from the Orinoco river in Venezuela.

Amphotericin-B belongs –to the family of a Polyene macrolide and effective against the fungal mucosal inflammation and Aspergillosis, and has limited activity against the protozoa and does not have antibacterial action. The drug release deoxy cholale in the blood whereas, the remnants of Amphotericin-B in the plasma constitute more than 90% in the plasma that sour rounds protein mainly beta lipoprotein. The effectiveness of anti-fungal A mphotericin – referred to the part interact with the sterol and ergosterols in the plasmal membrane of the fungus such interaction with the. cell membrane leads to the formation of

holes or channels which leads to an increase in the permeability of the membrane and allow the passage of small molecules, furthermore, oxidative damage was observed in the cells of the fungus. (Goodman and Gilmans, 2001)

Demarie *et la.(*1994) observed the accumulation of Amphotericin-B in the liver and spleen, they also noted that liver toxicity is not considered the principle of the drug.

Johnson *et la.,.*(1998) indicated that the pain associated with injection as in the back, abdomen or sometimes the chest usually occur in patients who take the first few does.

Bekersky *et al.*(1999) mentioned that doses above 10 mg/kg that rarely given to patients, causing no deaths, where the doses of 8 mg/kg were highly toxic in dogs.

The therapeutic dose of Amphotericin-B ranged from 0.5 to 0.6 mg/kg b.wt. with daily slow intravenous injection from the most fundamental effects of Amphotericin-B, fever, chills, and sometimes an increase in respiratory rate, reduction of blood pressure or hypersensitivity, also the metabolic requirements may be disturbed. And Amphotericin-B decrease the production of hemoglobin, causing anemia of red blood cells and which supressed the following treatment slowly and sometimes a lake of platelets and white blood cells was observed also furthermore, tissue damage sustained to the renal tubules and disturbance in renal function were observed by Goodman and Gilmans, (2001).

Vogelsinger *et al.* (2006) indicated that the levels of Amphotericin-B were high in the liver and spleen, followed by kidney, heart muscle and brain, while concentrations of lipo- Amphotericin-B were high in the lung.

2. Materials and Methods

1-Costus: (Fig1)

Costus extract was prepared by heating 100 ml of water, then 10 grams of powdered roots of Costus, were added boiled for 3 minutes, covered and stored in a refrigerator at a temperature of less than 25 (Adzu *et al.,*2001).

The animals were treated by this extract orally using stomach tube.

2-Amphotericine-B:

The Molecular formula of Amphotericine-B is $C_{47}H_{75}NO_{17}$, and its Molecular weight is 924. 084.Ten ml of sterile water were added to the vial content for injection.

Thus, the concentration will be 0.1 mg/ ml and kept at temperture2-8^{o}C.

The drug was given to the animal by Alveli intravenous injection(Goodman and Gilmans,2001)

3-Aspergillus niger:

A. niger was obtained from the National Research center Microbial and Natural Products- Arab Republic of Egypt. Cultivation and preparation of spore suspension were carried out in microbiology laboratory-faculty of Education-Jeddah.

A – Preparation of spore suspension of *A.nger*:

The spore suspension of *A.niger* was prepared from two days old culture grown on solid Sabourad dextrose medium by adding 5ml of phosphate buffer solution according to the method adopted by Hadecek, and Greger, (2000).

The animals were treated by diluted spore suspension(1x10-3) by distillation in the nose.

B-Determination of dry weight of *A. niger:*

The conical flasks containing different concentrations of costus extract (5, 10, 15 ml/ 100ml of culture medium) in addition to control flask were inoculated with 5 mm diameter discs from 6-days old cultures of *A.niger*, incubated at 25-2^{o}C then filtered after three days.

C- Antagonistic tests:

To test the antifungal of activity Costus against the pathogenic fungus, a disk of *A.niger* (5mm diameter) was inoculated in the center of sterilized petridish containing sterilized solid sabourad dextrose medium. Thereafter, 0.25 ml of water extract of costus were added in a hole (5mm diameter) was obtained in the solid medium. Control samples were obtained without the addition of Costus extract to the fungus. The dishes were incubated at 27^{o}C. Six replicates were done for each treatment (Sastry and Dutta,1961)……

4-Experimental Animals:

In this study, 90 male Albion rats *Rattus norvegicus,* of 21 days old (the age of post-weaning) and weights ranging from (50-60 grams), were obtained from the King Fahd Center for Medical Research of the University Of King Abdul Aziz.

All transactions injection and autopsy, taking of samples, dying of histological sections were carried out in laboratories for Faculty of Education for girls in Jeddah.

Experimental animals were divided into the following groups:

First Group:

This group comprised 30 control rats, they were given distilled water through mouth during the experimental period.

Second Group:

This group included 10 rats treated with the spore suspension of *Aspergillus niger* and injected with a dose (0.4mg/ kg b.wt.) by nasal distillation, six doses every other day for two weeks and then two weeks after the last dose.

Third Group:

This group comprised the animals treated with extract Costus and the 40 mice are divided into 4 subgroups as follows:

A- Comprised 10 rats treated only with Costus extract by oral administration with a dose of (0.2mg /kg), daily for three weeks and then examined.

B- Included 10 rats treated only with. Costus extract and given at dose of (0.4mg/ kg) by mouth, daily for three weeks, and then explained.

C- This subgroup comprised 10 rats treated with *A.niger* spore, suspension(0.4mg/ kg) by distillation in the nose six doses every other day for two weeks and then treated after Two weeks at a dose of Costus extract (0.2mg/ kg) by though daily oral injection for 10 days and then examined.

D- and included 10 rats treated with *A.niger* a dose of (0.4mg/ kg) through Distillation intranasal six doses every other day for two weeks and then treated after two weeks with Extract Costus at a dose (0.4mg/ kg) by though daily oral injection for 10 days and then examined.

Fourth Group:

This group comprised 10 rats infected with *A.niger* (0.4mg/ kg) by nasal distillation, six doses every other day for two weeks. Then they treated with Amphotericin-B(0.2mg/ kg) by daily intravenous injection for 10 days and then explained according to Vanetten *et al.* (2000).

Note that he has been appointed effective doses, and also different concentrations of all transactions after Make several initial test results for each of the materials used in this research.

Histological and ultrastrustural studies:

At the end of the experimental period, rats were killed by chloroform, lung samples were taken, cut and placed in various fixatives in order to conduct the histological and ultra structural studies.

Dehydration, clearing, paraffin embedding and cutting of samples (3micron diameter) were carried out. The sample painted by haematoxylin and Eosin stain and blue Toluedin for histological studies. (Lillie, 1965).

Ultrastructure studies by using the transmission electronic microscope (Robenson *et al.,* 1987).

3. Results and Discussion

I-microbial studeis:

As appeared from fig. (2), the extract of Costus plant showed highly effective ant agonistic activity against the pathogenic fungus *A.niger*. As shown, inhibition zone surrounded the holes containing the Costus extract and prevented the fungal growth compared to the control sample at which the fungal growth occupied the hole petridish.

Also, it could be observed the sharp decrease in the biomass of the fungal mycelia as a result of Costus extract treatment Thus, the inhibition of fungal growth reached 97.2% at conc. of 15% of Costus extract compared to the control sample (Fig.3).

II: Histological and ultrastructure studies:

First Group (Control samples) :

Histological studies:

The respiratory system consists of the lungs and air ways, which in turn are divided into respiratory passages. In the embryo in a manner similar to the emergence of glands of the dermis, and Bronchi the lungs protected inside the respiratory girdle. Lung consists mainly of and Bronchlotes and alveoli in addition to the blood vessels nerve fibers and a few connective tissue. The pulmonary components the external side to are alveoli as follows: primary Bronchi, Secondary Bronchi, Tertiary Bronchi, Bronchioles, Terminal bronchioles, Respiratory bronchioles, Alveolar Ducts, Alveolar Sacs and Alveoli.

Bronchioles:

It is composed of inner layer in the form of clear by which have ciliated columnar epithelial cells with few goblet cells and smooth muscle layer surrounding the original Lamina propria and surrounded from the outside by Adventitia. There are no glands or cartilage, near the Bronchioles near of the bronchlole there is a branch of pulmonary artery, and surmounted by Alveoli (Fig. 4).

Terminal bronchioles:

Terminal bronchioles are Lined with cuboidal ciliated epithelium without goblet cell and replaced by Alkosip Clara cells (tall columnar cells with apical secretory granules), thick layer of smooth muscle thin plate and surrounded from the outside by adventitia terminal Bronchioles have no cartilage or glands and accompanied by branch of the pulmonary (Figs. 5 & 6).

Respiratory bronchioles:

Respiratory Bronchioles are lined by cubidal ciliated epithelium lining the epithelial cells and the number of few of non-ciliated cells called Clara cells which replace goblet cells they are surrounded by smooth muscle layer which surrounded by the elastic fibrous connective tissue and each respiratory Bronchiole divided into several alveolar ducts which turn end by alveolar sac which open into several alveoli.

Alveoli & Interalveolar septa:

Each Alveoli include pocket open on one side of the route of alveolar septum composed of three components: epithelial surface, connective tissue and blood vessels. Epithelial layer which constitute the continued lining vesicle include two types of cells (Fig. 7): The first type includes most of the liposome surface area that are heavily covered with cells called squamous cells lining the vesicles P1 air pneumonia (cells lining follicular) (Pneumocytes) type II

epithelial cells known as P2 cells lining respiratory alveoli (Pneumocytes) which occupy a small proportion of about 5% of the liposome surface. Capillaries form most of alveolar septum and that branch and intertwine to form basket-like arrangement on each alveoli. Based on the cells lining the convex side the membrane while the basal cells lining the vascular poetry and the concave side next to the red blood cells within the capillaries. The barrier between alveoli composed of capillaries surrounded by vesicular network formed of elastic collagen fibers with of squamous epithelium of neighboring on both sides of the capillary network, also contains barriers acinar vesicular pores, which allows some movement of air between neighboring vesicles. Thickening of collagen fibers and elastic fibers around alveolar opening and constitute the support of the lung tissue (Garner and Hiatt, 2006).

Ultrastructural studies :

Bronchioles:

Terminal bronchioles are lined by cubic ciliated epithelial cells or Non-ciliated cells. The ciliated cells move the secretions and prevent the arrival of particles into the throat whereas the non-ciliated cells called Clara cells are the main characteristic of the terminal bronchioles secretory function.

Clara cells have a head like a dome for filling the region apical granules secretory dense irregular shape of the article kleikoz Ominoclaekanat that may maintain the lining of the bronchi. Clara cells contain mainly large mitochondrial and the base contain a nucleus and a rough endoplasmic reticulum with patches of glycogen, and the tops of the network are smooth and a Golgi apparatus which is not developed (Fig. 8) (Johnson,1991)

The Clara cells have three important functions:

1. Produce components of Surfactant which kleikozominokleikinat.
2. Serve as producing cells. They are capable of dividing (Figures 17,18) and replacing affected cells.
3. Contain a device capable of enzymatic detoxification of harmful substances.

Alveoli:

Vesicles are the unit of the basic structure and function of the lung. A vesicular wall called the Interalveolar Septum consists of five major types of cells:

- (Endothelial cells) 30%.
- (Type I Pneumocytes) 80%.
- (Type II Pneumocytes) 16%.
- (Interstitial cells) include (Fibroblasts) (Mast cell) 36%.
- (Alveolar macrophages) 10%. (Young *et al.,* 2000).

Endothelial cells:

Endothelial cells of the blood vessels capillaries are very thin and can be easily suspected with cells of type I squamous P1, which is based on cells of the first type found on the convex side of the membrane base while the cells lining the vascular noodles on the concave side and next to any red blood cells within capillaries,

the endothelial cells of vessels are related and non-perforated, gather the nuclei and other organelles to help areas in the cell to be very thin in order to increase the efficiency of gas exchange. A very notable appearance in the flat parts of cytoplasm is the presence of many Pinocytic vesicles as in (Fig 9).

Type I Pneumocytes P1:

Most of the surface area is covered with a large Squamous alveolar cell, called cell lung Type I Pneumocytes (P1) Alveolar lining cells.) Alveolar lining cells are highly squamous splayed and essentially intertwined in a lined vacuum vesicular, these cells have a strong nucleus distinct from the nuclei of cells lining the capillaries, cytoplasm has organelles such as Golgi apparatus and endoplasmic reticulum and mitochondria that accumulate around the nucleus and thereby reduce the thickness of the blood-air barrier, leaving large areas of cytoplasm free of organelles.

Cytoplasm contains in the thin parts Pinocytic vesicles, which play a role in the transformation of turnover of surfactant and the removal of pollutants in small plywood from the outer surface, ribosomes are organized in bundles within the cytoplasm, even in most areas and vulnerable cells contain pneumoniae P1 contacts applied Occluding junctions working to prevent the leakage of tissue fluid into the vacuum vesicular. The main role of these cells is to provide a thin barrier which douches gases easily

Type II Pneumocytes P2:

Among squamous cell lung P1, there are spherical cells called (Type II Pneumocytes P2), or Surfactant cells. There are connections associated between the cells of P1 and P2.Usually P2 is at the sites which combines the alveoli composing angles that have almost cubic shaped cells. These cells have a large spherical Nucleus large,Foamy cytoplasm vesicular shape due to the presence lomellar bodies that contain concentric or parallel plates. Studies in Chemistry tissues showed these Lomellar bodies contain phosphorous laminate fat from Type Dipalmatoyl Lecithin and Kleikozominoclaekanat and proteins.

These objects are considered as granular glands (Surfactant) which is working to reduce the strain on the surface tension of the cells in the alveoli and prevent closure of lung during exhalation and reduces the amount of energy required to emphysema during inspiration. Also, cytoplasm contains cells of type II,

the mitochondria and a rough endoplasmic reticulum, free ribosomes, a well-formed Golgi apparatus and and a number of vesicular objects and Cytossmes. P2 is surrounded by basal membrane and a small proportion of its surface exposed to vesicular vacuum and showing microvilli related to secrete a material called Surfactent as in (Fig.10)

Interstitial cells:

The barrier in the alveoli contains cells of a connective tissue as Fibroblasts (Fig, 11) and Mast cells (Fig, 12). The interstitial fibroblasts compose collagen fibers and elastic fibers which are intensified in the barrier of the vesicular to strengthen the fabric of visceral lung.

Alveolar macrophages:

The lung Contains big phagocyte cells which are launched in the liposome blanks and barriers. They are active cells and have a surface of irregular shape because of the false underfoot movement. They are Aptlaip and secretory cells, where it protects and cleans the surface of epithelial vesicles from the microbial damage and particles of organic and inorganic dust by ingesting the exotic materials.

Macrophages contain many secondary lysosomes and fatty drops. Also the number of reflect the size of the phagocytosis process of those cells. In the fullness of macrophages with dust, they transmit either to the top of respiratory tree, (Fig.13).

Second Group:

Histological studies :

Rats treated with the suspension of *Aspergillus niger* (0.4 mg/kg).

Examination of the hisological sections of rats infected with the fungal suspension indicated a significant loss of the normal lung structure due to the degeneration and distortion as a result of infection dominated by the appearance of aggregations of inflammatory cells mainly lymphocytes around the bronchioles (Fig.8).

Also, some bronchioles loosed its normal shape due to distortion, dilatation, lysis of the internal lining layer and fibrosis (Fig.9).

The epithelial tissue of the distorted respiratory bronchioles showed irregular structure with congested blood vessels with accumulation of red blood cells further, significant increase in the thickness of alveolar walls and interalveolar septum with narrow lumen of some of them (Fig. 16).

Respiratory bronchioles:

Respiratory bronchioles are lined by cuboidal ciliated epithelium and few non ciliated cells called Clara cells instead of goblet cells. They are surrounded by smooth muscular cells which surrounded by elastic fibrous connective tissue. Each respiratory bronchioles divided into several alveolar ducts which in turn ended by alveolar sac which open in several alveoli.

The examination of the semi-thin section revealed that the vesicles were distorted with thick wall with narrow lumens which is known as collapse phenomenon (Fig.17). Also, the number of pulmonary cells of type P_2 increased in the alveolar wall. As a result of the sever injury, the rats lung were subjected to closure of the alveoli and decrease in the number of terminal bronchioles with their distortion which led to the disappearance of Clara cells. Bennett (1979) indicated that *Aspergillus* grow in the human tissue through the respiratory passages such as trachea or pneumonic cavity which is known as fungal pulmonary diseases or Aspergillosis.

Trachea and lung can be infected by fungal spores which affect pleura, air can enter to it through the rupture of alveoli (Al gamas and Dia Eldin, 1983).

Asperigllosis was known to affect the respiratory system causing fungal pneumonia and funal bronchitis, also the inhaled fungal spores cause hypersensitivity (Abduel Hamid, 2000).

Luther *et al.* (2007) mentioned that the pulmonary macrophage cells constitute an important part of early immune defense mechanism against the *Aspergillus* infection, accordingly, engulfing of fungal spores is essential to git rid of the infection.

The clotting of blood in the vessels is considered the responsible factor for the death resulting from *Aspergillosis* (Lai *et al.,* 2007).

Ultrastrustural studies :

A group of rats infected with fungus *Aspergillus niger* dose (0.4 mg / kg). When examining the sectors of the ultra-structure of the lungs of rats

A.niger infected dose (0.4 mg / kg) and found many areas covered with large amounts of bleeding. A study of alveoli increased histopathological changes in comparison with those of prior infected rats. These changes were in the small number of cells P1 surrounded by bleeding and the occurrence of cytoplasm decomposition, while the number of cells P2 largely increase which led to a lack in air spaces in the tissue, which reduces the area of gas exchange in the tissue.

Those changes weren't only in the number, they exceeded the internal structure of P2 cells, it was observed a significant increase of laminate objects compared to former infected rats and the small number of mitochondria, where an analysis occurred to its customs and interior membranes as well as to the nucleus division and a large increase in the size in some of the P2 cells or an atrophy of the nucleus and the distorted in other cells, and reduction of the Golgi apparatus.

As a result of infection, the macrophages largely increased in the vesicular which confirms the infection. Also lysosomes frequently appeared to communicate to the process of phagocytosis of these

defensive cells (Figures18,19,20). Studies carried out by (Parke, 1994) have shown that when the animals' lung tissue is expose to jet fuel particles, it melts in a lining epithelial liquid of the bronchioles and primarily aimed at macrophages,Clara cells and P2 cells, where all these cells partially absorb it.

(Ochs *et al.,* 1999) reported that pneumonia servictnt is one of the active ingredients in a defense mechanism against pneumococcal infections. Both the (Hawgood, 1997; Gunther *et al.,* 1999) pointed that the importance of pulmonary surfactant change comes through its prevention of the collapse by reducing the surface tension of the vesicles.

Hays *et al* (2003) studied the impact of Propulsion jet fuel -8 on the respiratory efficiency of male rats' lung weighing (200-250 gm). He studied the fine structure of vesicular cells type II P2, when rats exposed 24 hours for doses (0,5 0.1 0.0, 1,5 mg / g) many of the micro-structural changes appeared. Mostly an increase in the number of laminate objects, while high dose (1,5 mg/g) was decomposed and P2 cells lost their normal shape.

Alokail & Alarifi, 2004 tested the use of the Arab incense smoke on rats' lungs. He found that it causes changes in the histology of the intra- barriers and the blood tissue in treated animals. The study showed the presence of infections which appeared in lymphocytes and plasma cells in the connective tissue around the vascular and inherent class to some bronchi, while the liposome barriers that surround bronchioles increased in thickness and filled with lymphocytes, acidic and neutral cells. the use of incense smoke caused an increase in the pneumonia damage which is represented in the increase numbers of macrophages liposome that spread in the lbranchema as well as the increase in size, so-called Hyperplasia phenomenon.

Alarifi *et al.,* 2004 and others studied the minute structural changes in the fabric of rats' lung exposed to the smoke of the Arab incense. One of the main results of the study was minute synthetic changes for most organelles, and appeared to be evident the lung tissue the Hyperplasia phenomenon for the vesicular cells and increase in presence of neutral cells in the infected alveoli with a crash and changes in interstitial cells and necrosis in the liposome, as seen for the deposition of collagen fibers in the liposome walls. The study conducted by the electronic microscope confirmed that the use of incense smoke made minute synthetic changes in the alveoli that led to a lack of respiratory efficiency

Both (Harrison, 2004 and Davidson, 2006) mentioned that the disease of the lung indicates an inflammation in the lungs due to fungal infection. It is accompanied by pneumonia and pain in the chest concentrated in the shoulder or top abdominal wall, difficulties in breathing and dry cough at the beginning of the disease, most often accompanied by green or yellow or rust-colored expectoration. It might be smelly with symptoms of fever, loss of appetite, the diagnostic procedure observed violation, such as chronic pulmonary air sacs, small bronchi, odema and an increase in the number of acid and pulmonary blood cells. Luther *et al.,* 2007, mentioned that pulmonary macrophages are an important part of the early immune defense against *Aspergillus* infection and thus the process of fungal spores ingestion is a necessary condition to eradicate it.

Third Group:a.b

Histological studies :

Rats treated with Costus extract (0.2 and 0.4 mg/kg).

The histological examination of lung section of rats treated with Costus extract (0.2 and 0.4 mg/kg) showed that the lung tissue appeared with its traditional normal structure and most of its components appeared in the normal position.

As appeared in (Fig. 21) air bronchioles present in regular lung tissue and consists of internal epithelial layer which in turn consists of columnar ciliated cells with goblet cells in between, with extended invaginations inside the cavity and surrounded by muscular layer in addition to the blood vessel.

It could be observed that the terminal bronchi structure is similar to that of the air bronchi (Figs. 22,23). However, the internal epithelium consists of ciliated Cuboidal cells and characterized by the presence of Clara cell which replaced the globlet cell. The cavity of the terminal bronchi connected to that of the respiratory bronchi which in turn branched to several vesicular channels ended with alveoli and alveoli air sacs. As appeared from (Fig.24) the wall of alveoli if p1 and p2 cells. With complete absence of callapse phenomenon.

C- Rats treated with *Aspergillus* suspension (0.4 mg/ kg) then treated with costus extract (0.2 mg/ kg).

The microscopic examination of the histological lung structure of rats infected with fungal suspension (0.4 mg /kg) then treated with Costus extract (0.2 mg/ kg) revealed the restoration of the interstitial tissue of lungs nearly its normal structure and appeared devoid of degeneration (Fig. 25).

The histological sections of lungs showed the air bronchioles characterized by normal and regular structure, the internal epithelium consists of clear invaginations around nearly regular cavity. Also, the alveolar sacs showed regular structure at which the alveolar consists of normal walls with clear distinguished cells.

D- Rats treated with *Aspergillus* suspension (0.4 mg/ kg) then treated with costus extract (0.4mg/ kg):

Examination of lungs of rats infected by *Aspergillus* suspension (0.4mg /kg) then treated with costus extract showed the positive effect of costus plant in the restoration of the lung tissue to its traditional and normal structure and the disappearance of the negative effects and pathological changes caused by fungal infection.

Fig. 26 showed well formed and regular air bronchioles in the treated rats compared to those of infected once.

The air bronchi characterized by internal epithelial layer consisted of ciliated columner cells with globlet cells arranged in folds inside well developed lumen. The blood vessels with regular walls were non-congested and located adjacent to air bronchioles. Furthermore, the air sacs appeared with regular walls characterized by clear P1 and P2 cells with complete absence of collapse phenomenon (Fig 27), The high prevalence of diseases, the side effects of some drugs in addition to its high cost have led to an increase in demand for use of natural product in pharmaceutical industry. Metwally (2005) indicated that costus plant prevent sputum, addresses common cold, costs and pleurisy pain, tetanus and provides protection against toxins and its adverse effects.

Costus arabicus was effective against chronic bronchitis and asthmas (Dutta *et al.,* 1960,& Sastry and Dutta, 1961).

Tsarong *et al.* (1994) indicated that the popular traditional uses of costus were to address lung inflammation, coughs, colds, ulcers and rheumatism. Recently, Habsah *et al.* (2000) confirmed the antioxidants and antimicrobial activities of costus.

Although many published evidences support the effectiveness of costus plant and safe use, little information were known about the active ingredients in the plant, its bioavailability. So, studies on the physiological pathways and pharmacological importance were needed to provide good entries for new pharmaceutical uses of the plant (Pandey *et al.,* 2007).

Costus is considered from the famous medicinal plants described traditionally specially in India, China and Korea.

The effectiveness of Costus aginst cancers, infections, liver hyperactivities was confirmed by Pandy *et al.* (2007).

Parekh and Chanda (2008) indicated that the Methanolic extract of Costus and Saussurea Lappa, belonging to the same family composite showed higher antifungal activity compared to the chemical antifungal Amphotericin B and fluconazle. Furthermore, methanolic extract of costus was very effective against three species of *Aspergillus* and the effect of the extract depend on the fungal species. Thus, low concentrations inhibit *A. Flavus* where, higher ones inhibit *A.niger*. In addition, extract of costus plant showed antioxidant activity due to the presence of polyphenols which inhibit the oxidative stress of free radicles (VijayalaKshmi and Sarada, 2008). This study was conducted to lest the effect of costus plant on the histological structure of lungs of rats infected with *Aspergillus*. The results revealed the effective effected of costus as natural and safe antifungal. Further medical and pharmacological studies were needed.

Ultrastructural studies :

(A - b) A group of rats treated with a dose of Costus extract (0.2 mg / kg -0.4 mg / kg). Examination with the electronic microscope for a sector of rats' lungs treated with Costus extracts dose (0.2 mg / kg - 0.4 mg / kg) showed that most of the components of the textile were natural developed position. The vesicular emerged thickness similar to what it was in the control samples, Also regularity of endothelial cells of the poetic shell, P1, P2 cells were well-formed where a cascade ideally capillaries in P1 cells in the vesicular wall. P2 cells seeming installing an internal regular and the nucleus of a clear and a number of objects laminate natural mitochondrial and endoplasmic reticulum clear (Fig., 28), as well as proven microscopic examination presence of mast cells are similar to the control samples (Fig., 29) and macrophages was characterized by a number of natural lysosomes (Fig., 30).

(C - d) A group of rats infected with *A.niger* dose of [0.4 mg / kg] treated with a dose of extract treatment[0.2 mg / kg - 0.4 mg / kg]: The exact examination for the infected rats' lung with *A.niger* dose (0.4 mg / kg) treated with dose (0.2 mg / kg - 0.4 mg / kg) confirmed the return of the natural appearance for thickness of the walls liposome and low number of P2 cells the optimum recovery to be installed, usually in terms of shape of the nucleus and the return of the mitochondria and customs of semi-natural appearance, also macrophages that have few lysosmes appeared as evidence of the decline in the process of phagocytosis resulting from the damage of injury (Figures, 31,32,33).

Due to the ineffectiveness of drugs which lost its original value in the treatment and their destructive effects on other intact, in addition to its high cost, we want to shed light on the wealth of the great field of prophetic medicine, which is honest, and the safest and most effective medicine to exist because it is originally from God The Almighty, where he says: (that is only a revelation revealed) Al-Star: Aya (4). Prohet Mohammad peace be upon him in the novel of Al-Bukhari from Umm Qais the daughter of Mahsen, she said: I heard the Prophet peace be upon him say: (use this Indian lute in which the seven heal: snort of its faces and generate tags).

Neuwinger, 1996, reported that species Aframomum (Zingiberacea) contain Quercetin and Kaempferol and both have the ability to prevent the growth of fungus, yeast and viruses also contain Syringic acid, which is an important and topical anesthetic for anti-Parkinson.

Also (Schmidt, 1999) found that the plant is rich in sesquiterpene lactones with the inhibitory activity and its ability to develop new drugs, especially used for the treatment of acute and chronic inflammation such as chronic arthritis. It is also the main component of the premium composite (Brahmyadi Ghanavati)) and the user as an officer for high blood pressure Rath *et al.,* 1999

Habsah *et al.,* 2000 reported that the methanolic and methanolic bilateral chlorine plant extracts species Zingibeeraceae including installment showed an antifungal activity of microbes and anti-oxidants where the methanolic bilateral chlorine extracts is stronger than methanolic extracts and interpreted so as to lower component terminals located in methanolic bilateral chlorine extracts (not polar) contributed towards increased activity for methanolic extracts (polar).

Both (Anjaria *et al.,* 2002;& Sriram *et al.,* 2004) say that the roots of abstracts of the premium is used as a treatment for asthma, bronchitis and a swollen abdomen and leprosy. Jeong *et al.,* 2002;& Cho *et al.,* 2004 mentioned that cynaropicrin and costunolide that contain premium is one of the factors used against cancer

A study conducted by (Tane *et al.,* 2006), he demonstrated that extracts and compounds (Aframomum,Zingiberacea), including the installment of vital activity of an anti-fungal toxins and cellular and anti-bacteria, parasites and viruses and anti-cholesterol increase. Pandey *et al.,*(2007) explained the uses of traditional Costus and proven its therapeutic effectiveness against cancer, ulcers, infections, liver protection activities. Magassouba *et al.,* 2007 Pointed out that active chemical compounds distinguish plants' extracts which are used in traditional medicine such as Costus that is responsible for the observed effect against bacteria. In the study conducted by (Vijayalakshmi & Sarada, 2008) on types of of Costus extracts, they verified that they contain Polyphenol and possession of an anti-oxidant activity shown in frequent presence of hydroxyl radicals, which works to quell the activity of free radicals.

The fourth group (G4): Rats infected by *Aspergillus* suspension 0.4 mg/ kg then treated with Amphotericin B.

Histological studies :

The lungs of rats infected with *Aspergillus niger* (0.4 mg/ kg) and treated with Amphotericin B generally showed deformed tissue as a result of infection. Microscopic examination showed irregular deformed air bronchioles with increased wall thickness of alveoli which filled with infiltration and bleeding (Figs. 34,35). Some of the bronchioles were continued with each others, while others loosed its normal shape inside deformed pulmonary tissue. Inflammatory invasion was detected on the epithelial layer of the bronchioles, with distorted blood vessel filled with bleeding (Fig.36).

Examination of the semi thin sections of terminal bronchioles did not show any detected improvement after amphotericin treatment where clara cells secretions filled the bronchiole lumen which lost its normal shape. Furthermore, the alveoli walls were highly thickened leading to atelectasis in some areas of the lung tissue (Figs.37,38)

Amphotericin -B showed dangerous side effects on brain and kidney. Toline and Raij (1988) treatment of rats with amphotericin –B caused renal toxicity lead to increase in the resistance of renal capillaries. Also Chavanet *et al.* (1992) comfirmed the occurrence of renal toxicity with lmg amphotericin /kg. Carlson and Condon (1994) indicated that 80% of patients treated with amphoterici B suffered from hyper nitrogenaemia. Wingard *et al.* (1999) observed that renal toxicity coused by amphotericin B- lead to disturbancein glomerular infiltration. Also, tissue damage of renal tubules and disturbance in lenal functions were detected with small doses of amphotericin (Walsh *et al.*, 1999).

Olson *et al.* (2006) tested the effectiveness of amphotericin B on rats infected with aspergillosis and other immune dificients non-infected.The observed high levels of blood urinary nitrogen in the non-infected rats, and degeneration in the renal tubules in the infected rats after drung treatment. Furthermore, amphotericin B caused encephalopathe and its daily repeated intravenous injection (0.5mg/kg) resulted in the appearance of amphotericin in plasma (1-105mg/ml) and in different body and few concentrations penetrated to cerebrospinal fluid (Blamaceda *et al.* 1994). Amphotericin was found to decrease hemoglobin production (anemia), platelels and white blood cells (Goodman and Gilman`s, 2001).

Nrajvar *et al.* (2004) indicated that amphotericin B up to 5mg/kg not sufficient for the treatment of rats from sever aspergillosis.

Amphotericin B with its different lipoid structures character zed by different, patterns of accumulation, thus it is accumulated in the lung tissue and after 24hours of treatment. whereas, liposomal amphotericin B showed more accumulation in the epithelial lining fluid (Groll *et al.,* 2006). In this

concern,Vogelsinger *et al.* (2006) indicated that the levels of accumulated amphoterici B were in the lungs of patients treated with colloidal amphotericin B than those treated with liposomal one.

Ultrastrustural studies :

A group of rats infected with a dose of *A.niger* (0.4 mg / kg) [and treated with Amphotericin - B: examination pointed out thorough the installation of histological lung, rats *A.niger* dose (0.4 mg / kg) and treated with Amphotericin - B continued negative effects of infection in spite of treatment with the drug, as there was no significant improvement after treatment with the drug Amphotericin - B cells pneumoniae P1 and P2, which appeared cells P1 atrophic and of abnormal nuclei, while cells P2 cells emerged frequently as there has been no improvement to their interior structures of the difference form the nucleus and increase number of objects laminate and the degradation of mitochondria, and capillaries appeared deformed and atrophic,also endothelial cells with the appearance of Sitoblazmi decomposition, The macrophages cells appeared large shapes, numerous lysosomes (Fig., 39,40,41)

Borgeat & Samuelsson, 1979; Turk *et al.* 1982;& Fels *et al.* 1982) mentioned that the neutral, acidic and macrophages cells in human lung epithelial cells, as well as Epithelial cells which are considered as materials for air passages are key sources for the production of Lipoxygenase enzyme. (Tolins and Raij, 1988) Found in the treatment of mice with Balomvutricin B an increase in renal vascular resistance as the result of renal toxicity. (Chavanet *et al.,* 1992) also stressed the emergence of renal toxicity when using Amphotericin - B for patients at 1 mg / kg.

According to both (Liu *et al.* 1995, 1996, 1997) when treated with the drug (DEC) of cases of asthma disturbance in physical activities as well as in the metabolic processes of Servictnt pneumonia. Nomura *et al.* 2001 found that drug (DEC) analyzes Lipoxygenase enzyme and thus inhibits macrophages liposome-producing enzyme by preventing the launch of its chemical activity.

The results observed from microscopic examination in a study carried out by Florencio *et al.* 2005 to determine the effect of drug diethy carbamazine (DEC) binary ethyl Cirbemaizin on the cells of the rats' lungs after treatment for 12 days and compared to Control Cells. That the P2 liposome cells of active nuclei by chromatin dense nuclei and clear vesicles secretory large number on the other hand, synthetic change wasn't noticed in P1 liposome cells, As macrophages have a number of ways to harmonize the cellular activity appeared with the nuclei of real chromatin and central nuclei(endossomes) in the stages of different growth (early and late) spread in the cytoplasm and these results support that the drug (DEC) plays a role in stimulating important cellular pathways which is likely to be related to therapeutic improvement in as a result for (asthma symptoms). Groll *et al.,* 2006, confirmed that Amphotericin - B and its different components are growing in the tissues of the lung and respiratory pulmonary macrophages bags.


الضابطة
المعاملة
بنبات بالقسط
A
B
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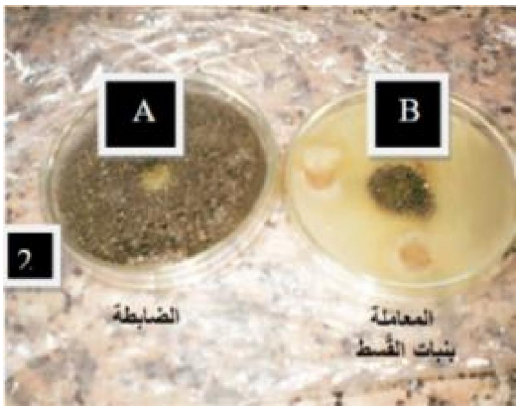
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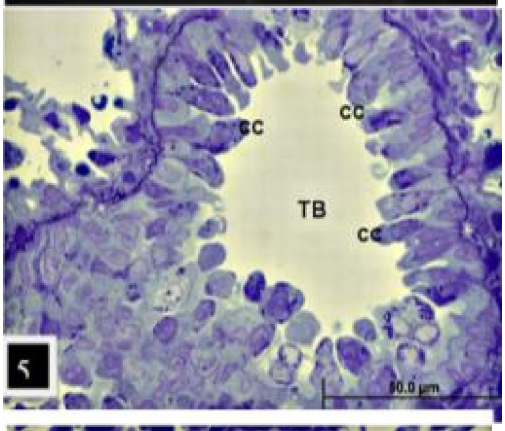
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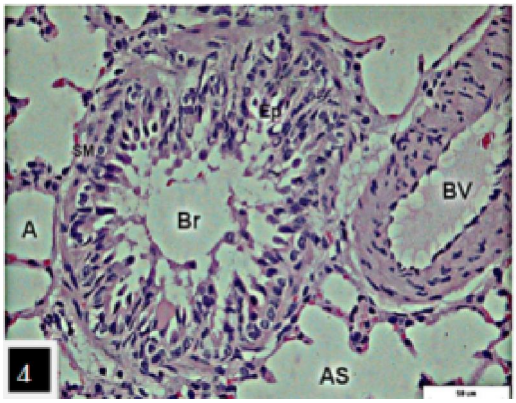
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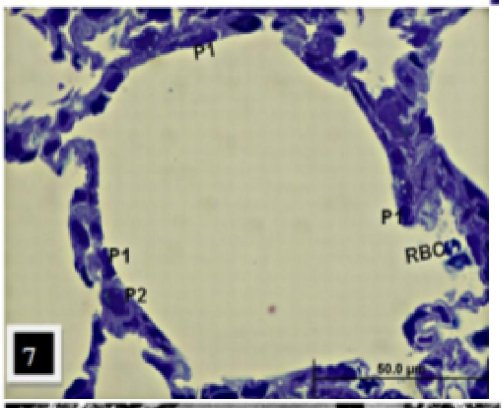
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P2
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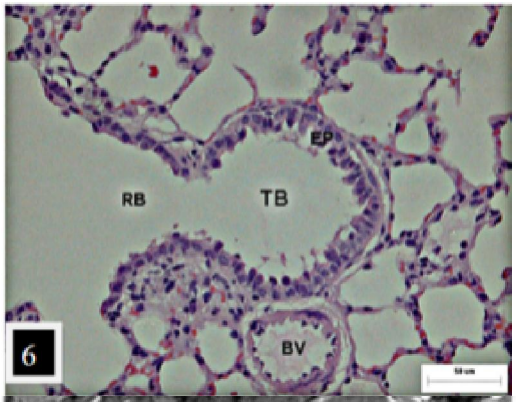
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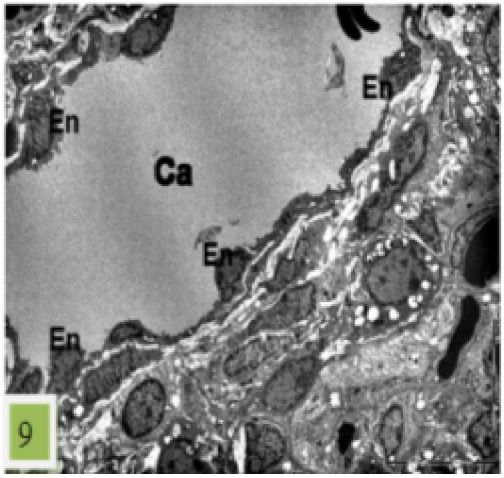
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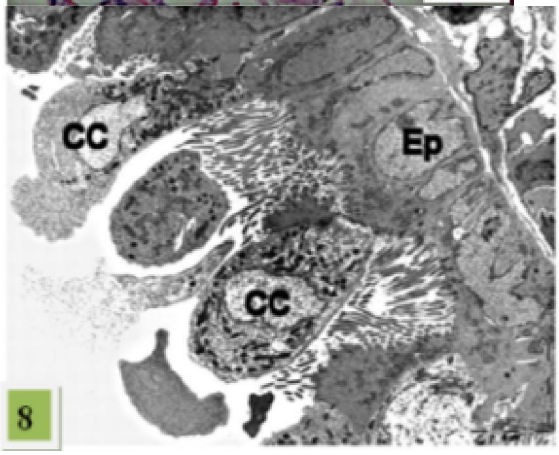
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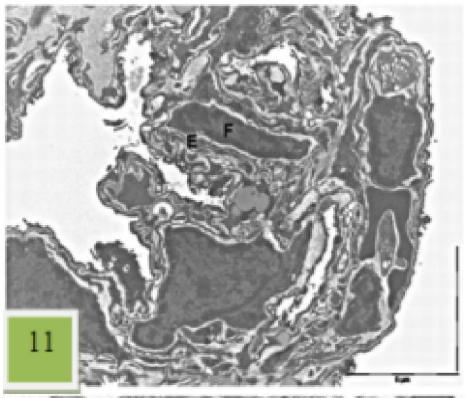
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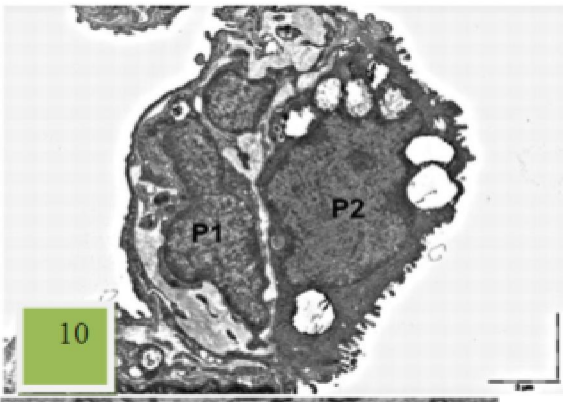
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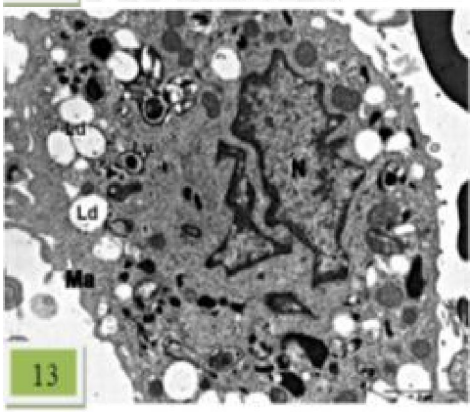
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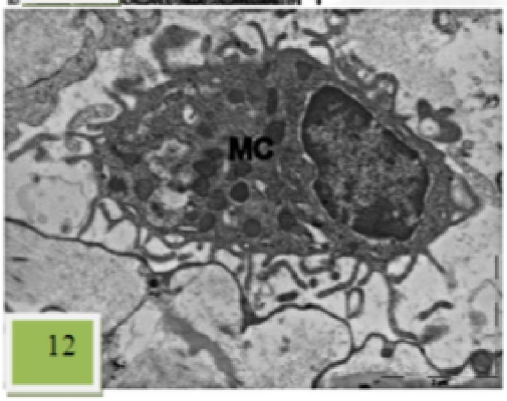
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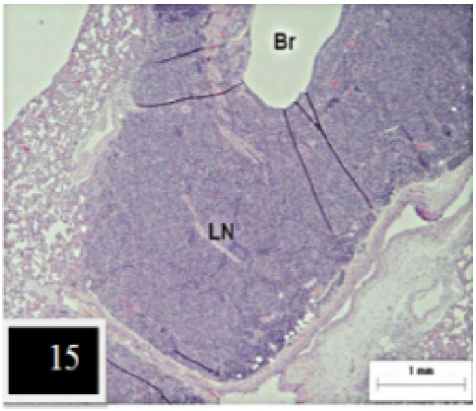
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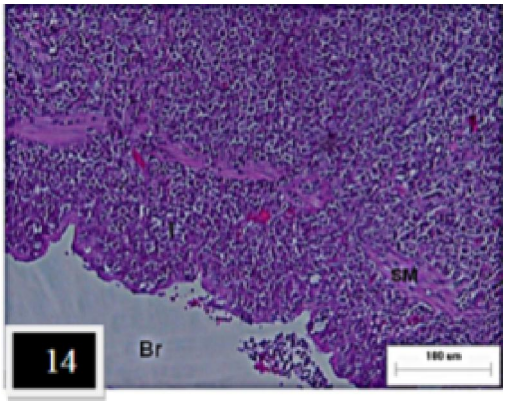
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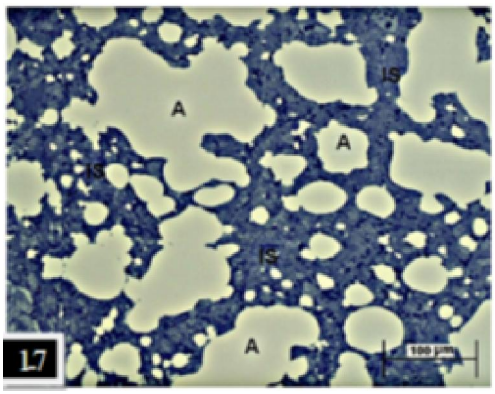
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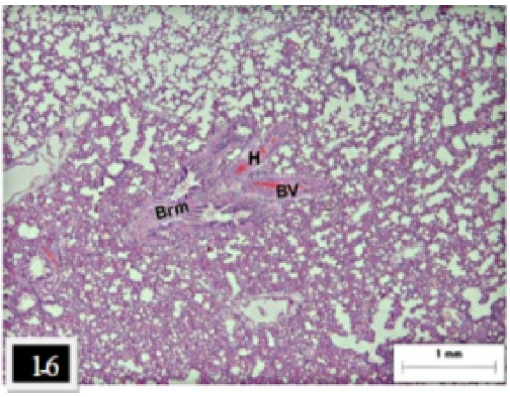
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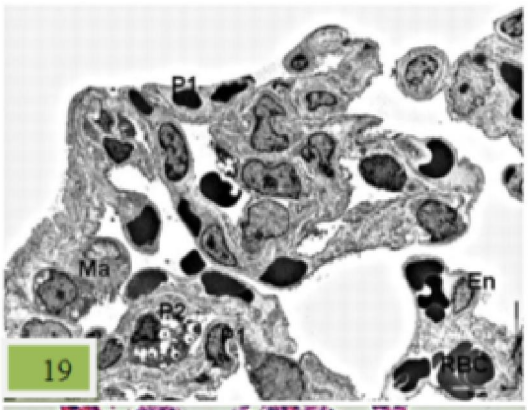
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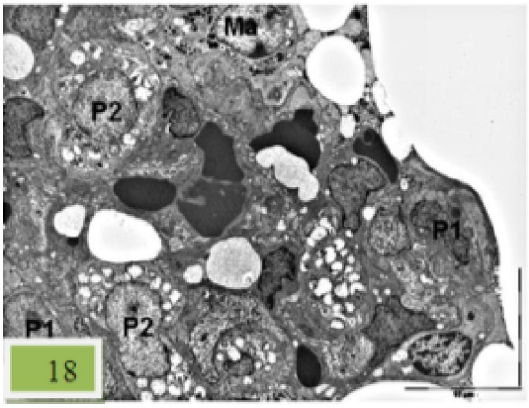
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P2
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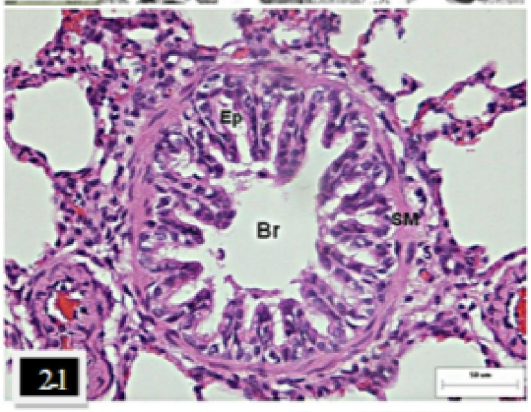
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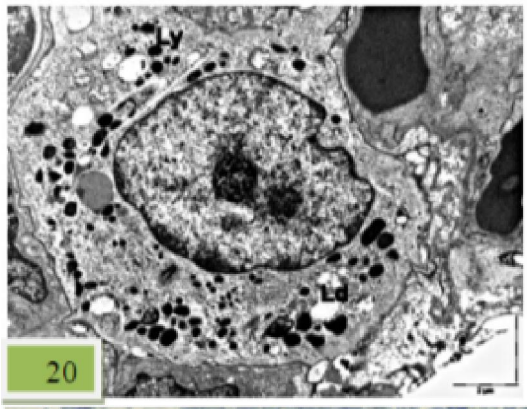
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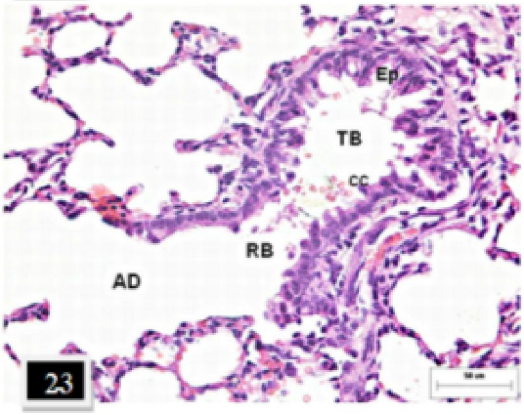
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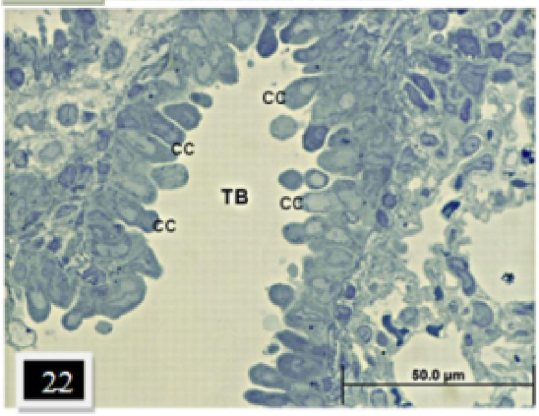
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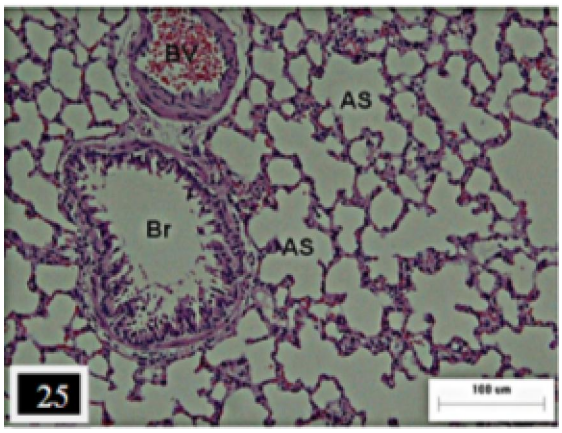
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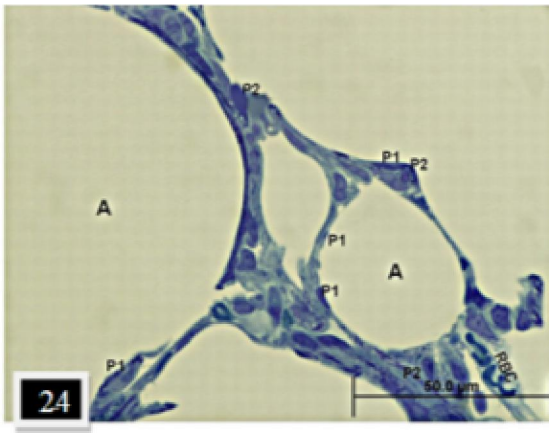
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P1
A
P1
P1
P2
RBC
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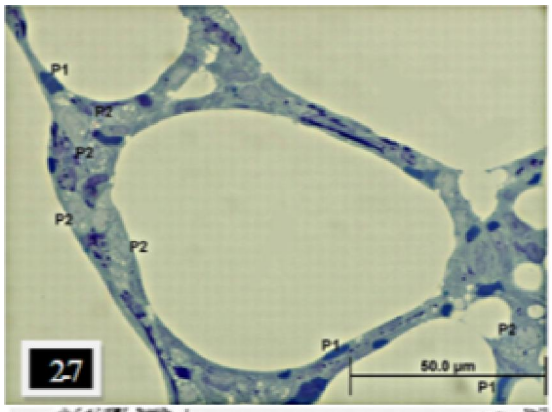
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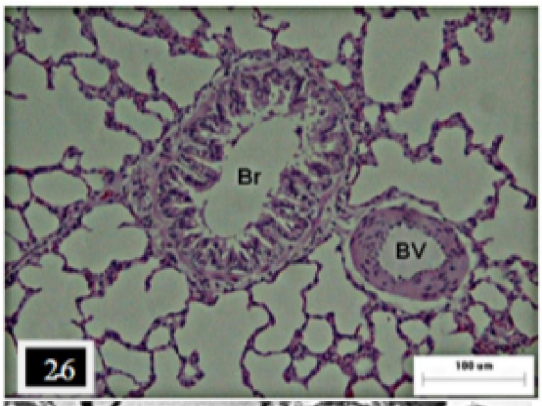
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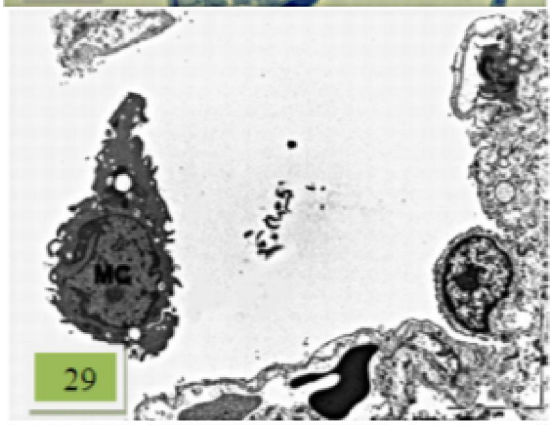
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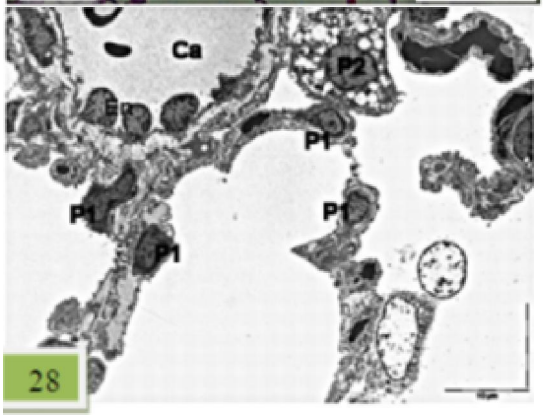
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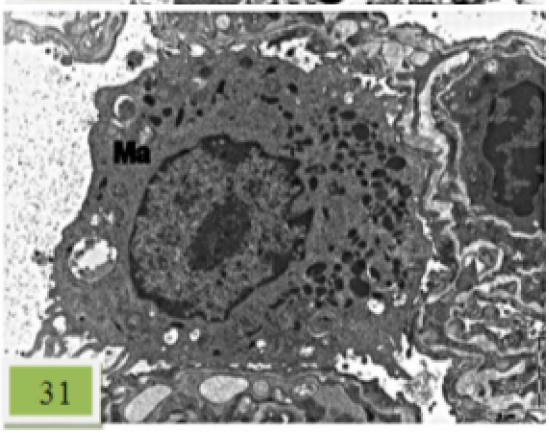
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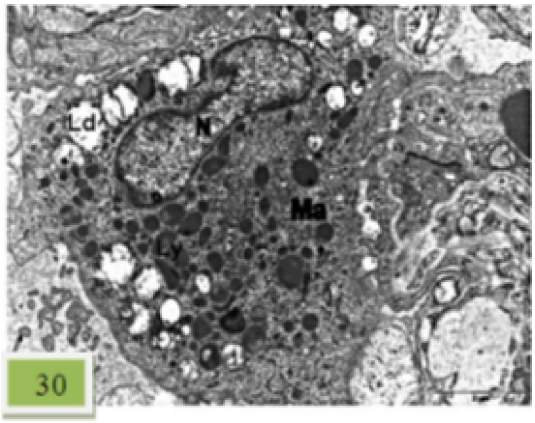
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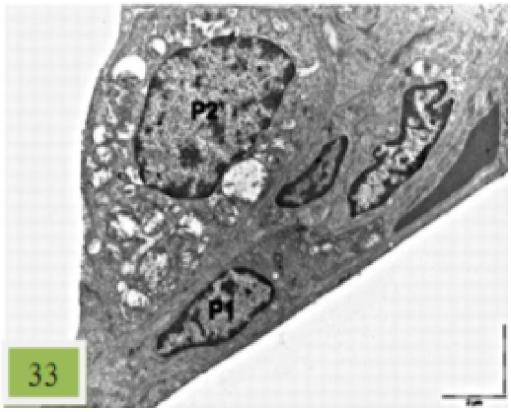
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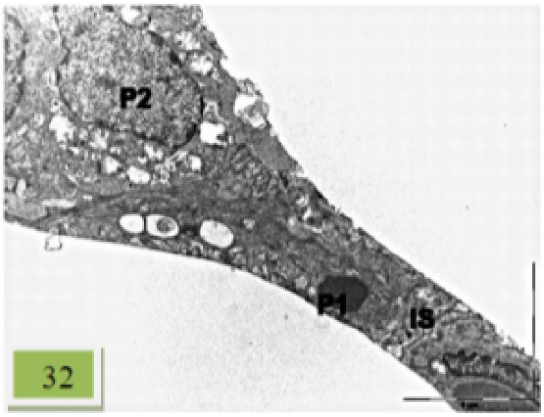
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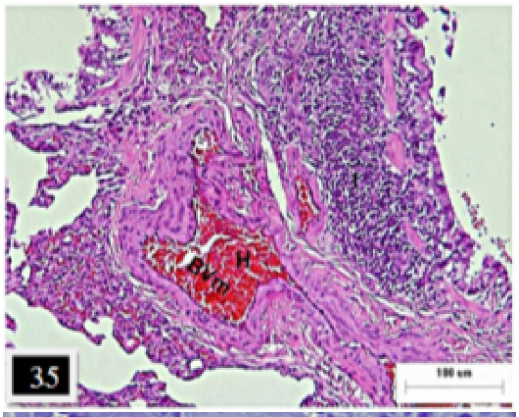
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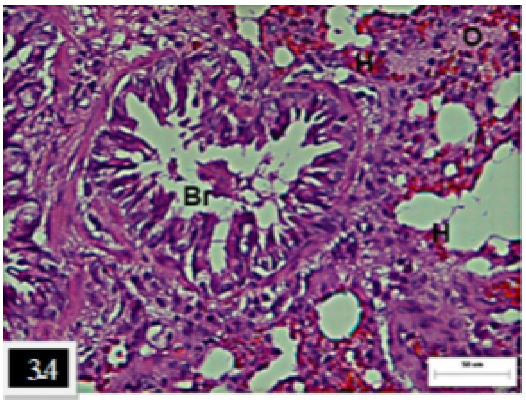
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H
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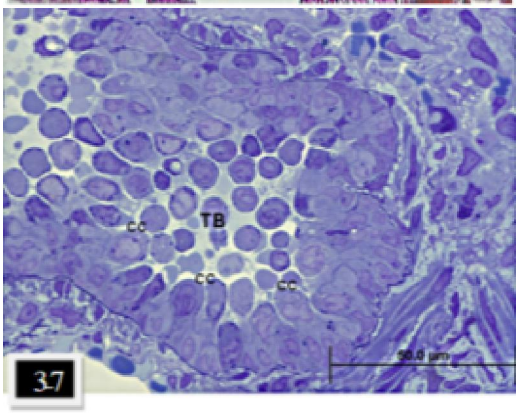
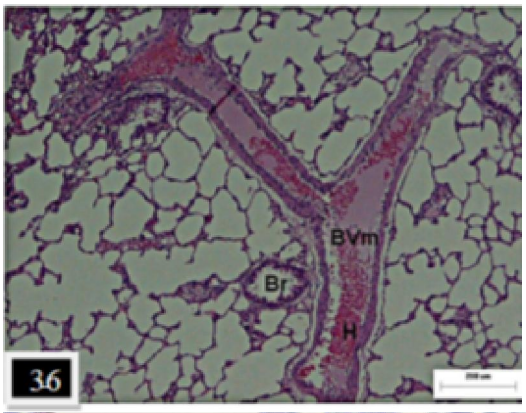
H
34


CC
TB
CC
CC
37


BVm
Br
H
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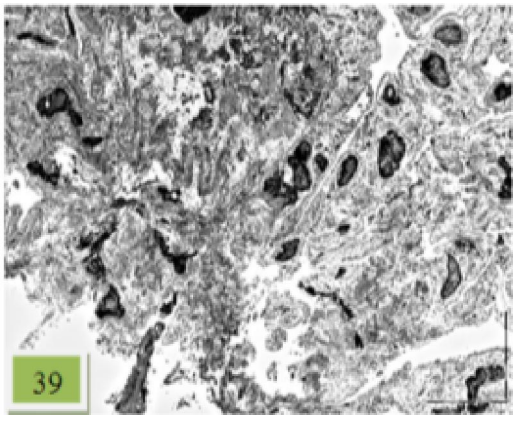
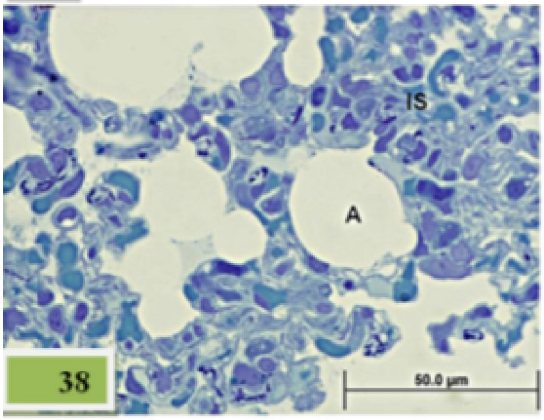
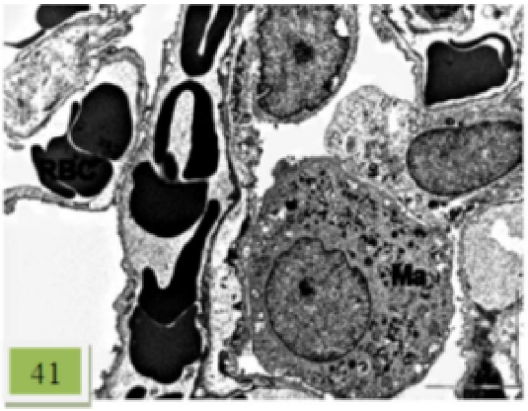
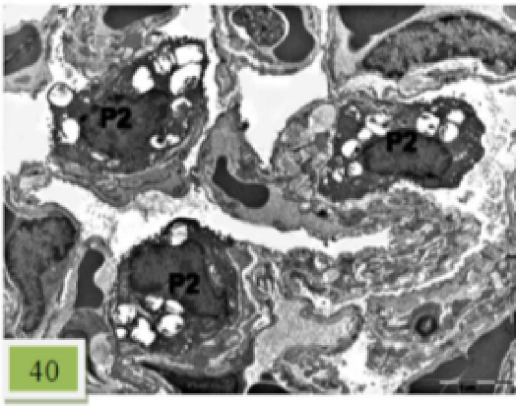

39


IS
A
38


RBC
Ma
41


P2
P2
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40

Fig (1): Roots of Costus Plant.
Fig (2): (A), Control group, the fungal growth occupied the hole Petridish. (B), Inhibition zone surrounded the holes containing the costus extract and prevented the fungal growth.
Fig (3): The sharp decrease in the biomass of the fungal mycelia a a result of costus extraet treatment (A), Control and (b) costus treatment.
Fig (4): Section of rat lung of control group showed bronchiole, (S M) Muscle layer, (EP) epithelial layer, (B v) Blood vessel, (A S) Alveolar sac and (A) Alveoli. (H&E) stain.
Fig (5) : semithin section in rat lung of control group showed, (E P) epithelial layer, (T B) terminal bronchiole and (C C) clara cells. Toluedin blue stain,
Fig (6): Section, in rat lung of control group showed, (TB) terminal bronchiole, (EP) epithelial layer, (RB) part of respiratory bronchiole and (BV) Blood vessel. (H&E) stain.
Fig (7): semithin section in rat lung of control group showed (P 1) type one, (P 2) type two of lung cells. (RBcs) red blood cell. Toluedin blue stain.
Fig (8): Electron section in rat lung of control group showed ciliated epithelial cells (EP) in terminal bronchioles and non-ciliated cells, clara cells (CC).x 2600.
Fig (9):Electron section in rat lung of control group showed the cell (En) lining the vascular noodles on the concave side of capillaries (Ca). X 1950.
Fig (10): Electron section in rat lung of control group showed lung cells p□ and p□.x 1450.
Fig (11): Electron section in rat lung of control group showed the cells of a connective tissue a Fibroblasts(F). x 4600
Fig (12): Electron section in rat lung of control group showed Mast cells (MC). X7900.
Fig(13):Electron section in rat lung of control group showed phagocyte cells (Ma) with its nucleus(N),lysosomes(Ly) and fatty drops (Ld).X 7900.
Fig (14): Section in rat lung of infected group with *A.niger* (0,4 mg/kg) showed epithelial layer (Ep) was degenerated in bronchiole (Br) which around with inflammatory cells(I). H & E stain.
Fig (15): section of rat lung of treated group with the suspension of *Aspergillus niger* (0, 4 mg/kg). Showed lysis of the internal lining layer and fibrosis in (Br) bronchile. (H & E). stain.
Fig (16): Section of rat lung of treated group with the suspension of *A.niger* (0,4 mg/kg). Showed (H) congested blood vessels, significant increase in the thickness of the alveolar walls with narrow lumen. (H & E) stain.
Fig (17): Semithin section in rat lung of treated group with the suspension of *A. niger* (0,4 mg/kg). Showed (A) alveoli with thick walls which is known as collapse phenomenon. Toluedin blue stain.
Fig (18): Electron section in rat lung of infected group with *Aspergillus niger* (0, 4 mg/kg). Showed increase number of P□ cells and decreas in P□ Cell.x1950.
Fig (19): Electron section in rat lung of infected group with *A.niger* (0, 4 mg/kg). Showed red blood cells (RBC) and an atrophy of the nucleus of P□ Cells. X1450. Fig (20): Electron section in rat lung of infected group with *A.niger* (0.4mg/kg).showed the macrophage with lysosomes(Ly). X5800.
Fig (21): Section in rat Lung of treated group with costus extract (0.4 mg / kg). Showed air bronchiole present in regular lung tissue (Br), muscular layer (SM) and (EP) epithelial layer. (H & E) stain.
Fig (22): Semi thin section in rat lung of treated group with costus extract (0, 4 mg / kg). Showed Clara cells (CC) in terminal bronchiole (T B).Toluedin blue stain.
Fig (23): Section in rat lung of treated group with costus extract (0, 4 mg / kg). Showed clara cells (CC) in terminal bronchiole (TB) and respiratory bronchiole (RB). (H & E) stain.
Fig (24): Semi thin section in rat lung of treated group with costus extract(0,4mg / kg), showed alveoli (A) and p□,p□ cells. (p□,p□). Toluedin blue stain.
Fig (25): Section in rat lung of infected group with the suspension of *A.niger* (0,4mg /kg). And costus exctract(0,4mg/ kg). and costus extract (0.2 mg/kg) Showed (Br) air bronchiole, Blood vessel (Bv) and alveolar sacs (AS) in regular structure.(H&E) stain.
Fig (26): Section in rat lung of infected group with suspension of *A. niger* (0, 4 mg/kg). And treated with costus extract (0,4 mg/kg). Showed air bronchiole (Br) in well formed and regular blood vessel (Bv) with regular wall and noncongested.(H & E)stain.
Fig (27): Semi thin section in rat lung of infected group, with suspension of A. niger (0, 4 mg/kg). And treated with costus extract (0, 4 mg/kg). Showed alveoli with clear p□ and p□ cells. Toluedin blue stain.
Fig (28): Electron section in rat lung of treated group with costus extract (0, 4 mg/kg) showed regularity of lung cells p□ and p□. X1450.
Fig (29): Electron section in rat lung of treated group with costus extract (0, 4 mg/kg) showed mast cell(Mc) x3400.
Fig (30): Electron section in rat lung of treated group with costus extract (0, 4 mg/kg).showed macrophage (Ma) with fatty drops (Ld) and lysosomes(Ly). X7900
Fig (31): Electron section in rat lung of treated group with *A.niger* (0,4 mg/kg) and treated with costus extract (0.2 mg /kg) showed macrophage(Ma). X5800.
Fig (32): Electron section in rat lung of infected group with *A.niger* (0,4 mg/kg) and treated with costus extract (0.2 mg /kg). Showed lung cells P□ a nd P□.x4600.
Fig (33): Electron section in rat lung of infected group with *A.niger* (0, 4 mg/kg) and treated with costus extract (0, 4 mg /kg). Showed lung cells P□ and P□. X 5800.
Fig(34,35): Section in rat Lung of infected group with suspension of *A.niger* (0,4 mg/kg) and treated with Amphotericin B. Showed irregular deformed air bronchiole (Br) with increased wall thickness of alveoli which filled with infiltration (0) and bleeding (H).(H & E) stain.
Fig (36): Section in rat lung of infected group with suspension of *A.niger* (0,4mg/kg) and treated with Amphotericin B. Showed distorted blood vessel filled with bleeding (Bvm). (H & E) stain.
Figs (37,38): semithin sections in rat lung of infected group with suspension of *A.niger* (0, 4 mg/kg) and treated with Amphotericin B. Showed clara cells (CC) secietions filled the terminal bronchiole lumen (T B). Toluedin blue stain.
Fig (39): Electron section in rat lung of infected group with *A.niger* (0, 4 mg/kg) and treated with Amphotericin – B showed increased wall thickness of alveoli. X1450.
Fig (40): Electron section in rat lung of infected group with *A.niger* (0, 4 mg/kg) and treated with Amphotericin – B showed lung cells P□ with increase number of objects laminate. X3400.
Fig (41): Electron section in rat lung of infected group with *A.niger* (0, 4 mg/kg) and treated with Amphotericin – B showed macrophages (Ma) around the capillaries (Ca) filled with red blood cells (RBC). X3400.

References

1. **-Adzu,B.; Amos,S.; Dzama,S.; Wambebe,C.; and Gamaniel, K.,(2001):**Effect of *Zizyphus spina-christi* willd aqueous extract on the central nervous system in mice.*J. Ethnopharmacol*. 79(1):13-16.
2. **-Alarifi, S.A.; Mubarak, M.M. and ALokail, M.S., (2004):** Ultrastructural changes of pneumocytes of rat exposed to Arabian incense (Bakhour). Saudi Med J. 11, 1689-1693.
3. **-Alexopoulos,C.J. and Mims,C.W., (1979):** Introductory Mycology, 3rd Ed, P:632, Jhon wiley and sons Inc., New York.
4. **-Alokail, M.S. and Alarifi, S.A., (2004):** Histological changes in lung of wister albino rats (Rattus norvegicus) after exposure to the Arabian incense (Genus Boswellia). Annals of Saudi medicine. 4, 293-295.
5. **-Anjaria, J.; Dwivedi, S.; Desai, P.and Thaker, A., (2002):** Traditional healers. in Ethnovet Heritage. Indian Ethnoveterinary Medicine: An Overview. Eds. Anjaria, J.; Parabia, M. and Dwivedi, S. Pratik Enterprise, Ahmedabad. pps 17-42.
6. **-Balmaceda, C.M.; Walker, R.W.; Castro-Malaspina, H. and Dalmau, J., (1994):** Reversal of Amphotericin B related encephalopathy. Neurology.,44:1183-1184.
7. **-Bekersky, I.; Boswell, G.W.; Hiles, R.; Fielding, R.M.; Buell, D. and Walsh, T.J., (1999):** Safety and toxicokinetics of intravenous liposomal Amphotericin B (AmBisome) in beagle dogs. Pharm. Res.,16:1694-1701.
8. **-Bennett,J.E., (1979a):** Aspergillosis.In, Beeson, Mcdermott, and Wyngaarden`sCecil textbook of medicine, 15th ed.Saunders,W.B., Philadelphia, PP.546-547.
9. **-Bennett,J.E., (1980):** Aspergillosis. In,Isselbacher,Adams,Braunwald,Petersdorff, and Wilson`s Harrison`s principles of Internal medicine, Mcgraw-Hill, New york,PP.742-744.
10. **-Borgeat, P. and Samuelsson, B., (1979):** Arachidonic acid metabolism in polymorphonuclear leucocytes: effects of the ionophore A23187. Proc. Natl. Acad. Sci. U.S.A. 76, 2148-2152.
11. **-Carlson, M.A. and Condon, R.E., (1994):** Nephrotoxicity of Amphotericin B. J. Am. Coll. Surg.,179:361-381.
12. **-Chavanet, P.Y.; Garry, I.; Charlier, N.; Caillot, D.; Kisterman, J.P.; D`athis, M. and Portier, H., (1992):** Trial of glucose versus fat emulsion in preparation of Amphotericin for use in HIV infected patients with candidiasis. J. B.M.,305:921-925.
13. **-Cho, J.Y.; Kim, A.R.; Jung, J.H.; Chun, T.; Rhee, M.H.; Yoo, E.S., (2004)**: Cytotoxic and pro-apoptotic activities of cynaropicrin, a sesquiterpene lactone,on the viability of leukocyte cancer cell lines. European Journal of Pharmacology 92:85–94.
14. **-Cruz, G.L., (1965): Livro verde das plantas medicinaise industriais do Brasil, Belo Horizonte. Velloso S.A.**
15. **-Davidson., (2006):** principle and practice of medicine, 20th, Edition Churchill Livingston.
16. **-Demarie, S.; Janknegt, R. and Bakker-Woudenberg, I.A.J.M., (1994):** Clinical use of liposomal and lipid-complexed Amphotericin B. J. Antimicrob. Chemother.,33:907-916.
17. **-Dutta, N.K.; Sastry, M.S. and Tamhane, R.G., (1960):** Pharmacological actions of an alkaloidal fraction isolated from *Saussurea lappa* (Clarke). Indian Journal of pharmacy,P:22:6-7.
18. **-Fels, A.U.; Pawlowaki, N.A.; Cramer, E.B.; King, T.K.; Cohn, Z.A. and Scott, W.A., (1982):** Human alveolar macrophages produce leukotriene B_4. Proc. Natl. Acad. Sci. U.S.A. 79, 7866-7870.
19. **-Florencio, M.S.; Saraiva, K.L.A. and Peixoto, C.A., (2005):** The effects of diethylcarbamazine on the ultrastructure of lung cells in vivo. Tissue and cell. 37, 241-246.
20. **-Gartner,L.P. and Hiatt,J.L., (2006):** Color atlas of histology, 4thEd, Lippincott Williams & Wilkins, USA, P:235-254.
21. **-Goodman and Gilman`s (2001):** The Pharmacological Basis of Therapeutics.10th ed. Joel, G.; Hardman LEE E. Limbird, New york,P:1295-1299.
22. **-Groll, A.H.; Lyman, C.A.; Petraitis, V.; Petraitiene, R.; Sein, T.; Bacher, J. and Walsh, T.J., (2006):** Compartementalized intrapulmonary pharmacokinetics of Amphotericin B and Its lipid formulations. Antimicrobial agents and chemotherapy. P:3418-3423.
23. **-Gunther, A.; Schmidt, R.; Feustel, A.; Meier, U.; Pucker, C. and Ermert, M., (1999):** Surfactant subtype conversion is related to loss of surfactant apoprotein B and surface activity in large surfactant aggregates. Experimental and clinical studies. Am J. Respir care Med. 159, 244-251.
24. **-Habsah, M.; Amran, M.; Mackeen, M.M.; Lajis, N.H.; Kikuzaki, H.; Nakatani, N.; Rahman, A.A.; Ghafar, and Ali, A.M., (2000):** Screening of Zingiberaceae extracts for antimicrobial and antioxidant activities. J.Ethnopharmacology,72:403-410.
- **Hadecek, F. and Greger, H., (2000): ……**Phytochem. Anal. P: 137-147.**Cited from Kurtaran,**R.*et.al*.,(2005) *Journal of Inorganic Biochemistry 99*.
25. **-Harrison., (2004):** principles of internal medicine, 16th Ed, McGraw Hill company.
26. **-Hawgood, S., (1997):** Surfactant: composition, structure and metabolism in the lung. Scientific foundations Crystal, R.G.; west, J.B.; Barens, P.J.; Weibel, E.R.; editors. 2nd.ed. Philadelphia (PA): Lippincott-Raven; P:557-571.
27. **-Hays, A.M.; Lantz, R.C. and Witten, M.L., (2003):** Correlation between in vivo and in vitro pulmonary responses to jet propulsion fuel-8 using precision-cut lung slices and a dynamic organ culture system. Society Toxicologic Pathogy. P:200-207.
28. **Hoshino,H.;Tagaki,S.;Kon,H.;Shibusa,T.;Takabatake,H.; Jain, S.P., (1984):** Ethnobotany of Morni and Kalesar (Ambala,Haryana). J.Economic and Taxonomic Botany, 5:809-813.
29. **-Jeong, S.J.; Itokawa, T.; Shibuya, M.; Kuwano, M.; Ono, M.; Higuchi, R.; Miyamoto, T., (2002):** Costunolide, a serquiterpene lactone from Saussurealappa inhibits the VEGER KDR/FLK-1 signaling pathway. Cancer Letter 187:129–133.
30. **-Johnson,K.E.(1991):** Histology and cell biology,2nd Edn, *Williams & Wilkins* (USA),P:197-206.
31. **-Johnson, M.D.; Drew, R.H. and Perfect, J.R., (1998):** Chest discomfort associated with liposomal Amphotericin B: report of three cases and review of the literature. J. Pharmacotherapy., 18:1053-1061.
32. **-Lai, C.; Liaw,S.; Lee,L.; Hsiao,C.; Yu,C.; Hsueh,P., (2007):** Invasive pulmonary aspergillosis: high incidence of disseminated intravascular coagulation in fatal cases, Infection and Immunity,P:141-147.
33. **-Liu, M.; Wang, L. and Enhorning, G., (1995):** Surfactant dysfunction develops when the immunized guinea-pig is challenged with ovalbumin aerosol.clin. Exp. Allergy 25, 1053-1060.
34. **-Liu, M.; Wang, L.; Holm, B.A. and Enhorning, G., (1997):** Dysfunction of guinea-pig pulmonary surfactant and type II penumocytes after repetitive challenge with aerosolized ovalbumin clin. Exp. Allergy 27, 802-807.
35. **-Liu, M.; Wang, L.; Li, E. and Enhorning, G., (1996):** Pulmonary surfactant given prophylactically alleviates an asthma attack in guinea pigs.clin. Exp. Allergy 26, 270-275.

36. **-Luther,K.;Rohde,M.;Sturm,K.;Kotz,A.;Heesemann,J.; Ebel,F., (2007):** Characterisation of the phagocytic uptake of *Aspergillus fumigatus* conidia by macrophages, Microbes and Infection,P:1-10.
37. **-Magassouba, F.B.; Diallo, A,; Kouyat´e, M.; Mara, F.; Mara, O.; Bangoura, O.; A. Camara, A.; Traor´e, S.; Diallo, A.K.; Zaoro, M.; Lamah, K.; Diallo, S.;Camara, G.; Traor´e, S.; K´eita, A.; Camara, M.K.; Barry, R.; K´eita, S.; Oular´e, K.; Barry, M.S.; Donzo, M.; Camara, K.; Tot´e, K.; Vanden Berghe, D.; Tott´e, J.; Pieters, L.; Vlietinck, A.J.; Bald´e, A.M., (2007):** Ethnobotanical survey and antibacterial activity of some plants used in Guinean traditional medicine. Ethnopharmacology 114: 44–53.
38. **-Neuwinger, H.D., (1996):** African Ethnobotany: Poisons AND Drugs. Chapman & Hall, London.
39. **-Nomura, H.; Sato, E.; Konama, S.; Haniuda, M.; Kubo, K.; Nagai, S. and Izumi, T., (2001):** Histamine stimulates alveolar macrophages to release neutrophil and monocyte chemotactic activity. Lab. Clin. Med. 138, 226-235.
40. **-Ochs, M.; Nenadic, I.; Fehrenbach, A.; Albes, J.M.; Wahlers, T. and Richter, J., (1999):** Ultrastructural alterations in intraalveolar surfactant subtypes after experimental ischemia and reperfusion. Am J. Respir Crit Med. 160, 718-724.
41. **-Otero, R.; Nunez, V.; Jimenez, S.L.; Fonnegra, R.; Osorio, R.G.; Carcia, M.E. and Diaz, A., (2000):** Snakebites and ethnobotany in the northwest region of Colombia, Part∏: Neutralization of lethal and enzymatic effects of Bothrops atrox venom. J.Ethnopharmacology,71:505-511.
- **Olson, J.A.; Adler-Moore, J.P.; Schwartz, J.; Jensen, G.M. and Proffitt, R.T., (2006):** Comparative efficacies, Toxicities, and tissue concentrations of Ampotericin B lipid formulations in a murine pulmonary Aspergillosis model. American Society for Microbiology. P:2122-2131.
42. **-Pandey, M.M.; Rastogi, S. and Rawat, A.K.S., (2007):** *Saussurea costus*: Botanical, chemical and pharmacological review of an ayurvedic medicinal plant. J.Ethnopharmacology 110:379-390.
43. **-Parekh, J.; Chanda, S., (2008):** In vitro antifungal activity of methanol extracts of some Indian medicinal plants against pathogenic yeast and moulds., J. Biotechnology, 23:4349-4353.
44. **-Parke, D.V., (1994):** The cytochromes P450 and mechanisms of chemical carcinogenesis. Environ Health Perspect. 102, 852-853.
45. **-Rath, S.T.; Mishra, R. and Das, B.K., (1999):** Management of Raktavata vis-à-vis arterial hypertension with Brahmyadi Ghana vati. J.Research in Ayurveda and Siddha,20:29-46.
- **Robenson, D.G.; Ehler, U.; Herken, R.; Hermann, B.; Mayer, F. and Schiirman, F.W., (1987):** Methods of preparation for Electron Microscopy. Spring veragle, Berlin, Heide berge, Germany.
46. **-Sastry, M.S.; Dutta, N.K.; (1961):** A method for preparing tincture Saussurea.Indian Journal of Pharmacy 23:247–249.
47. **-Schmidt, T.J., (1999)**: Toxic activities of sesquiterpene lactones: structural and biochemical aspects. Current Organic Chemistry 3:577–608.
- **Sriram, S. Patel, M.A. and Patel, K.V., (2004):** Compendium on Medicinal Plants. Gujarat Agricultural University, Ahmedabad, 1st ed., India; pp. 1-116.
48. **-Tane, P.; Tatsimo, S.D.; Ayimele, G.A.; and Connoly, J.D., (2006):** Bioactive metabolites from *Aframomum* species., P:214-223.
49. **-Tolins, J.P. and Raij, L.,(1988):** Adverse effect of Amphotericin B administration on renal hemodynamics in the rat-Neurohumoral mechanisms and influence of calcium channel blockade. J. Pharmacol. Exp. Ther.,245:594-599.
50. **-Tsarong, T., (1994):** Tibetan medicinal plants, Tibetan medical publications. India.
51. **-Turk, J.; Maas, R.L.; Brash, A.R.; Roberts II, L.J. and Oates, J.A., (1982):** Arachidonic acid 15-lipoxygenase products from human eosinophils. J. Biol. Chem. 257, 7068-7076.
52. **-Van etten, E.W.M.; Stearne-Cullen, L.E.T.; Ten Kate, M.T. and Bakker-Woudenberg, I.A.J.M., (2000):** Efficacy of Liposomal Amphotericin B with Prolonged Circulation in Blood in Treatment of Severe Pulmonary Aspergillosis in Leukopenic Rats, AntimicrobialL agents and chemotherapy, p. 540–545.
53. **-Vijayalakshmi, M.A.; Sarada, N.C., (2008):** Screening of Costus speciosus extracts for antioxidant activity. Fitoterapia 79:197–198.
54. **-Vogelsinger, H.; Weiler, S.; Djanani, A.; Kountchev, J.; Bellmann Weiler, R.; Wiedermann, C.J. and Bellmann, R., (2006):** Amphotericin B tissue distribution in autopsy material after treatment with liposomal amphotericin B and amphotericin B colloidal dispersion. Journal of Antimicrobial chemotherapy. 57, 1153-1160.
55. **-Walsh, T.J.; Finberg, R.W.; Arndt, C.; Hiemenz, J.; Schwartz, C.; Bodensteiner, D.; Pappas, P.; Seibel, N.; Greenberg, R.N.; Dummer, S.; Schuster, M. and Holcenberg, J.S., (1999):** liposomal Amphotericin B for empirical therapy in patients with persistent fever and neutropenia-National institute of Allergy and infectious diseases mycoses study group. N. EngI. J. Med.,340:764-771.
56. **-Walsh, T.J.; Goodman, J.L.; Pappas, P.; Bekersky, I.; Buell, D. N.; Roden, M.; Barrett, J. and Anaissie, E. J., (2001):** Safety, Tolerance, and pharmacokinetics of high-dose liposomal Amphotericin B (AmBisome) in patients infected with Aspergillus species and other filamentous fungi: maximum tolerated dose study. American Society for Microbiology. P:3487-3496.
57. **-Whistler, R.L.; Bushway, A.A.; Singh, P.P.; Nakahara, W. and Tokuzen, R., (1976):** Noncytotoxic, antitumor polysaccharides, Advances in carbohydrate chemistry and biochemistry,32:235-275.
58. **-Wingard, J.R.; Kubilis, P.; Lee, L.; Yee, G.; Walshe, L.; Bowden, R.; Ainaissie, E.; Hiemenz, J. and Lister, J., (1999):** Clinical significance of nephrotoxicity in patients treated with Amphotericin B for suspected or proven aspergillosis. Clin. Infect. Dis.,29:1402-1407.
59. **-Young,B.; Lowe,J.S.; Stevens,A.; Heath,J.W., (2000):** Functional histology a text and colour atlas, 5th Ed, Sydney,P:222-236.

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