

THERAPEUTIC ACTION OF HAELAN 851®
ON RAT SILICOSIS

ABSTRACT: This paper describes the observed effects of Haelan 851, Platinum Formula, nutritional oral liquid on experimentally induced rat silicosis. The results showed that after nutritional supplementation with Haelan 851, Platinum Formula, oral liquid the content of lung collagen, the dry lung weight and the wet lung weight was decreased and the activity of SOD increased. There were obvious significant differences between the observed group and the silicosis group ($P < 0.01$). Pathological changes on lung tissue and cells under lighted microscopes, both Transmission Electron Microscope (TEM) and Scanning Electron Microscopes (SEM), showed that the damage of lung tissue, alveolar macrophage and type II cells in the observed group was slighter than in the observed group. The results indicated that Haelan 851, Platinum Formula, nutritional oral liquid might restrain the developing of silicosis and provide a therapeutic action on silicosis.

INTRODUCTION

During the development of silicosis, the damage increases the action of lipid peroxidation to generate free radicals, which may damage biological membranes and cause the further damage of alveolar macrophage. This process forms the malignant circulation of products that damage and causes the development of silicosis.^{1,2,3,4} Haelan 851, Platinum Formula, oral nutritional liquid contains abundant amino acids, varying vitamins, trace elements and other important dietary nutrients. Clinical studies have demonstrated that Haelan 851, Platinum Formula, oral liquid increases the immunity action of the mononuclear macrophage system and is beneficial in reducing body impairment.^{5,6} This paper studied the lung collagen, superoxide dismutase (SOD) activity and pathological changes observed, including the effects of nutritional supplementation with Haelan 851, Platinum Formula, nutritional oral liquid on the experimental rat silicosis.

METHODS AND MATERIALS

Forty five (23 male and 22 female) Winstar rats weighing 200 to 250 grams each were divided into three groups: Control Group, Observed Group and Silicosis Group. Except for the control group, one milliliter quartz suspension, which contains 50 mg quartz per milliliter, was injected into the lungs of the rats through the trachea to establish the experimental rat silicosis model. The quartz particles were more than 95% in free SiO₂ and 95% in smaller than 5 μ m in diameter particles.

Fifteen days after injecting the quartz suspension the observed group was given nutritional supplementation with Haelan 851, Platinum Formula, oral liquid for nine weeks (15 ml for each dose and six times per week).

The three groups of rats were observed for 18 weeks. The blood of the rats was obtained by cutting the tail and the method of Joenge's was used to assay the activity of SOD.⁷ The rats were killed by bloodletting of the abdominal aorta after being anesthetized. Lung tissue was treated with choramine-T and the method of colorimetric determination was used to measure the lung collagen.⁸ The samples of lung were prepared for electron microscope observation as follows: small tissue blocks were dehydrated through a series of alcohols and were then transferred to liquid nitrogen and freeze fractured; the tissues were rehydrated and fixed with OsO₄ using a ligand-mediated banding method; the tissue was again dehydrated, critical-point dried and coated with approximately 100 Å thickness and observed by Transmission, Electron Microscope (TEM) (JEM-1200EX, Hitachi); lung tissue slices, which were cut by rotary microtome, were stained with HE and Gomori for observation using an Olympus light microscope. Lung lavage was prepared for the observation by Scanning Electron Microscope (SEM) (JSM-35CP, Hitachi).⁹

RESULTS

1. After the rats were killed, the lungs were weighed, both wet and dry weight, and the collagen content determined. Table 1 shows that the wet weight, the dry weight and collagen content may increase after giving quartz particles, and these changes may be decreased by nutritional supplementation with Haelan 851, Platinum Formula, oral nutritional liquid.

Table 1 - Changes of the Wet Weight, The Dry Weight, and the Collagen Content of Lung (mg)

	<u>The Wet Weight</u>	<u>The Dry Weight</u>	<u>The Collagen Content</u>
Control Group	2590 ± 410	318.4 ± 106.6	37.55 ± 8.05
Silicosis Group	5510 ± 1070*	878.8 ± 142.4*	111.79 ± 25.16*
Observed Group	3480 ± 1330*#	584.1 ± 218.6*#	79.56 ± 28.34*#

* compared with control group P<0.05

compared with silicosis group P<0.05

2. The quartz particles might cause the decrease of the activity of superoxidate dismutase, Table 2 shows that there were obvious significant differences between the silicosis group and the control group. But, SOD activity was increased for those rats (Observed Group) in the group nutritionally supplemented with Haelan 851, Platinum Formula, oral nutritional liquid. No difference was found between the observed group and the control group. (Table 2)

Table 2 - Changes of Activity of SOD Among The Three Groups (U/gHb)

	<u>SOD</u>	<u>P Value</u>
(1) Control Group	2940.68 ± 358.32	(1) : (2) <0.01
(2) Silicosis Group	2373.62 ± 427.13	(1) : (3) >0.05
(3) Observed Group	3330.51 ± 714.89	(2) : (3) <0.01

3. Pathological changes, observed with the light microscopes, between the observed group and the silicosis group are listed in Table 3.

**Table 3 - Main Pathological Changes Between
Silicosis Group and Observed Group**

Silicosis nodules:

Number:	existing all lungs, more than 3 nodules in every low times visual field	In general, existing alone less than 3 nodules in every low times visual field
Size:	large	small
Dust particles:	a large number	less than in silicosis group
Cell type:	alveolar macrophage with fibroblast	alveolar macrophage but less fibroblast
Fibrosis:	serious	slight

Lung tissue:

alveolar wall:	increased thickness	in general being normal
damage:	severe	slight

4. Morphology in electron microscope (Fig 1-7)

Control group -- the alveolar cavities were clear and its wall was uniform; type II cells, which its villi were short and rough located at the side of pulmonary alveoli and appeared oval shaped and approximately 5u in diameter. Alveolar macrophage were often seen and its villi appeared flower-leaf like shaped or finger-like projections, approximately 10 u in diameter.

Silicosis group -- the alveolar cavity were small and its wall became thick and not uniform, a large number of type 2 cells could be seen, the villi on its surface fell and appeared to have honeycomb-like damage, alveolar macrophage became large (up to 15 u in diameter) and appeared various shaped. Most of the cells had structure damage and appeared villi falling, being smooth or honeycomb-like damage on cell surface, with biological membrane damage and losing the cell characteristics.

Observed group -- the alveolar cavities were intact, its wall became thick but uniform, type II cells located at the side of pulmonary alveoli, the villi were short and rough and distributed evenly. Some of alveolar macrophage became large (up to 15 u in diameter), some cells lost its villi, but, the honeycomb-like damage or smoothness on cell surfaces were rarely found.

DISCUSSION

Reduction of molecular oxygen to water with a concomitant oxidization of substrates is the major mechanism of energy generation by the process of oxidative phosphorylation. This function is achieved in mitochondria material by a controlled, graded, series of electron-transfer reactions. This process may produce harmful substances, such as O_2 and H_2O_2 .^{10,11,12} These compound increases may also be evoked by alveolar macrophage (AM) phagocytosis of injurious particles such as silica.^{13,14} The antioxidant enzyme -- superoxide dismutase (SOD) may prevent damage by O_2 of the cell structure and it's function.¹⁰ Inhaling of silica particles may cause the damage of alveolar macrophage by increasing the quantity of O_2 and H_2O_2 . The superoxidant dismutase as a scavenger of these compounds is non-protective when it is exhausted.¹⁵ In this study, the activity of SOD in the silicosis group was decreased after injecting the quartz particles into the lungs of the rats. But, the nutritional supplementation with Haelan 851, Platinum Formula, oral liquid increased the SOD activity. These results indicate that nutritional supplementation with Haelan 851, Platinum Formula, oral liquid may protect the cell from the damage of free radicals by increasing the SOD activity.

The damage of alveolar macrophage caused by the inhaling of silica particles may play a part in the development of silicosis.^{1,2,3} The changes in lung tissue and cell morphology may explain the damage degree directly. Alveolar macrophage is an important part of the lung defense system. Its intact membrane structure keeps the cell's stability. When the quartz particles are injected into the lung, the lung defense system was aroused and caused the number of alveolar macrophage (AM) to increase. The phagocytosis of quartz particles by the alveolar macrophage (AM) may cause the changes of its surface and the damage of the cell's structure and function: villi falling and atrophy, appearing smooth or hole membrane damage in it's surface, finally losing the cell characteristics.¹⁶ Under observation with a Transmission Electron Microscope (TEM), the main changes were the damage of membrane structures, especially lysome and mitochondria. But Haelan 851, Platinum Formula, oral liquid stabilized the membrane structure to lessen the damage of both the lysome and mitochondria, finally, the development of silicosis might be restrained. The results of the observations have shown that the damages to the lung tissue, type II cells and alveolar macrophage in the Haelan 851 nutritionally supplemented observed group were less than in the silicosis group, which indicates the Haelan 851, Platinum Formula, oral liquid provided a therapeutic action on silicosis.

The wet weight, dry weight and collagen content of the lungs decreased in the observed group. As a final conclusion of lung fibrosis, this may explain the degree of fibrosis induced and further prove the therapeutic action of nutritional support with Haelan 851, Platinum Formula, oral nutritional liquid in cases of silicosis.

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Fig. 1
Type II cell under TEM in control group X5000

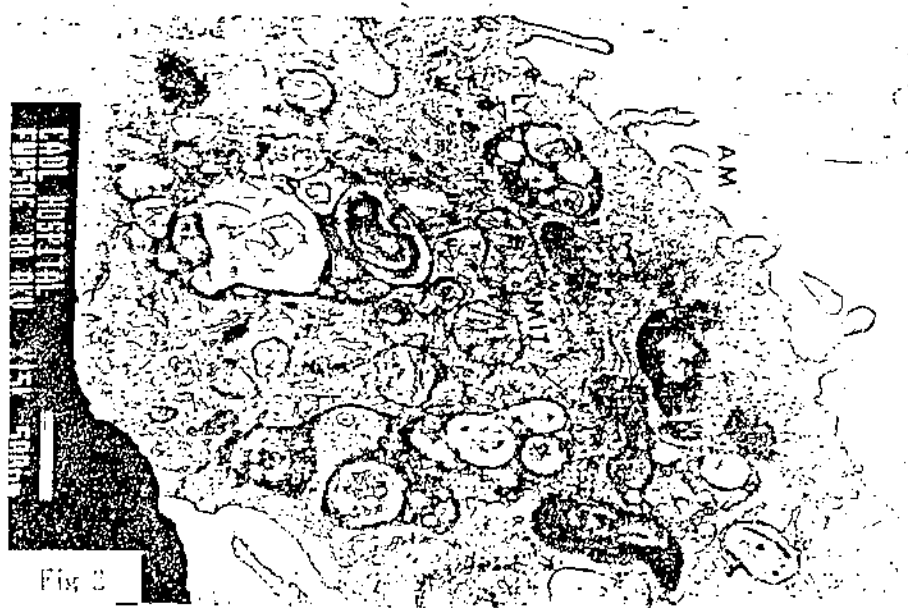


Fig. 2
AM under TEM in observed group, the membrane structure of lysosome and mitochondria are intact, the secondary lysosome can be found. X15000

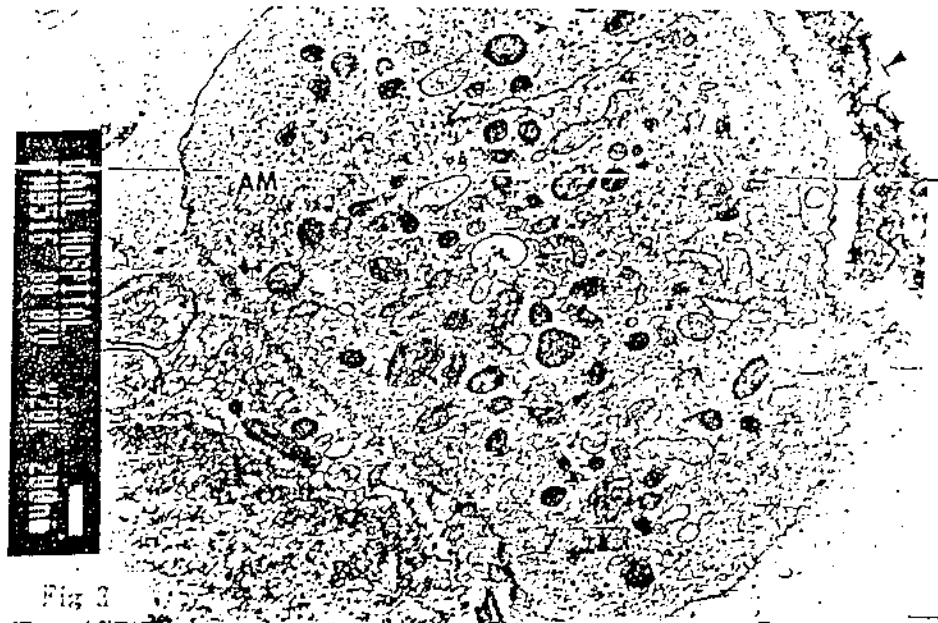


Fig.3
AM under TEM in silicosis group, the damage of villi and membrane of lysosome and mitochondria can be found X 20000

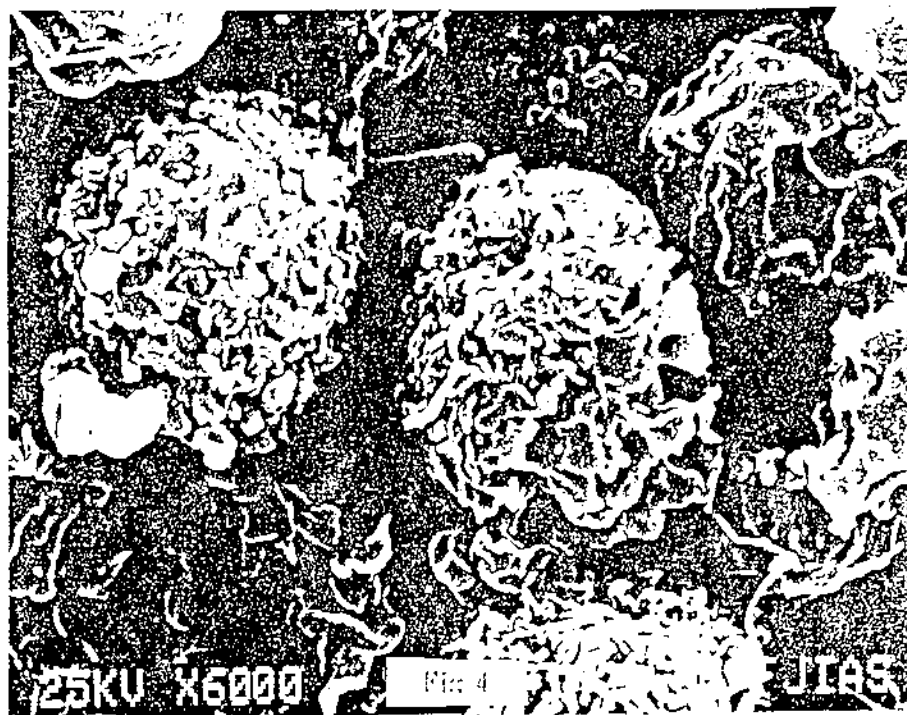


Fig. 4
AM under SEM in control group, flower-leaf-like and finger-like projects are found on its surface. X6000



Fig 5
AM under SEM in observed group, the flower-leaf-like and finger-like projects are found on its surface. X6000

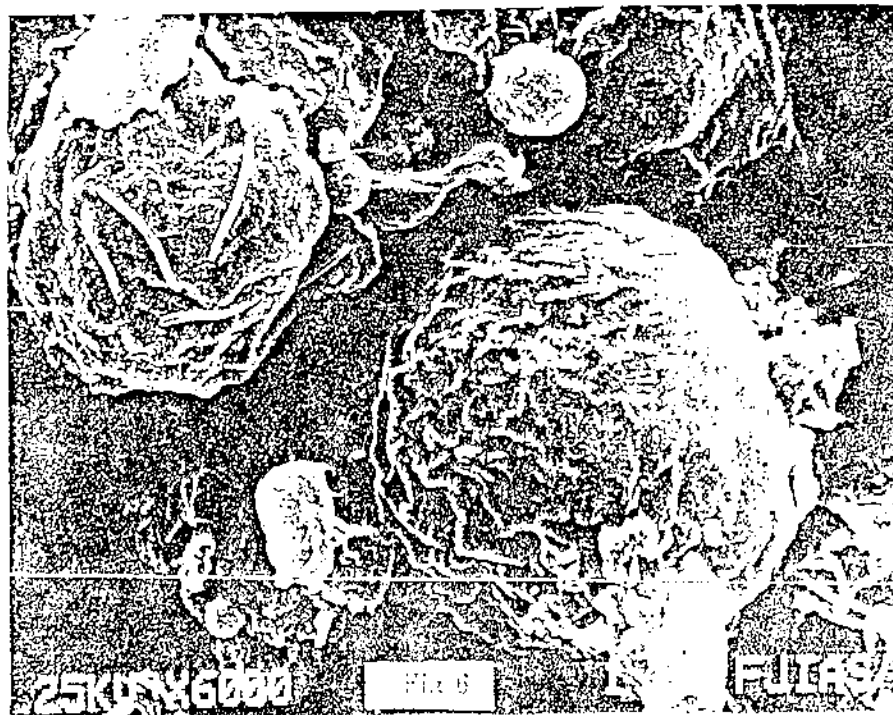


Fig. 6
AM under SEM in silicosis group, the villi fall and its surface is smooth. X6000

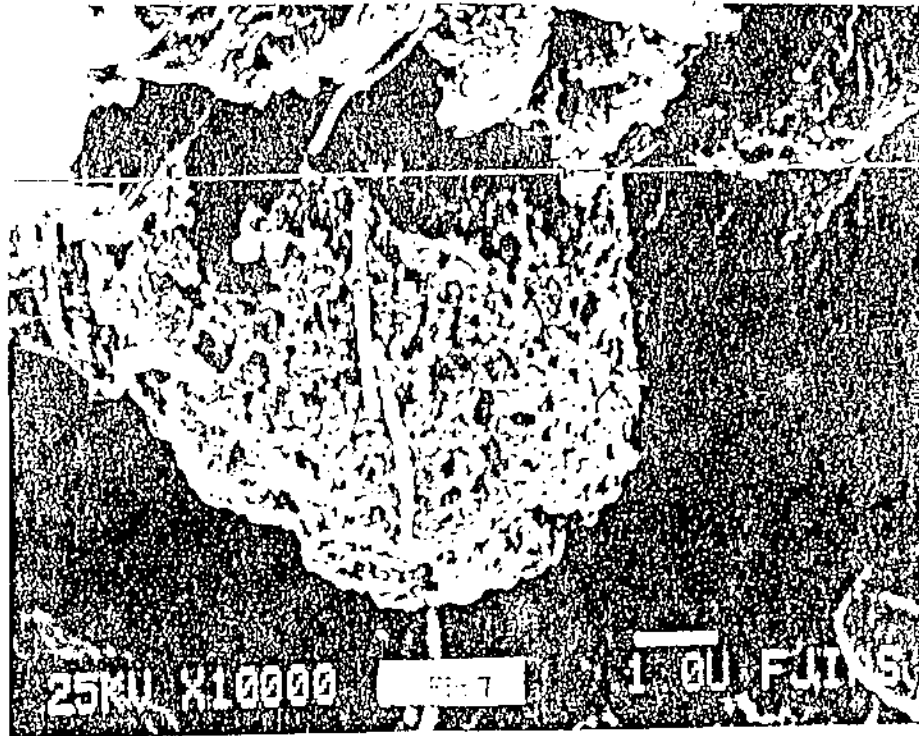


Fig.7

AM under SEM in silicosis group. Honeycomb-like
damage is found on its surface. X10000

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