

**Histological and Immunohistochemical Study to
Evaluate the Effects of Metformin versus Green
Tea Extracts on Bleomycin Induced Lung
Injury In Male Albino Rats**

Thesis

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Histology

By

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Summary

Bleomycin is an important chemotherapeutic agent that is most commonly associated with lung injury.

This study aimed to investigate the effect of metformin and green tea on bleomycin induced lung injury.

Forty eight male albino rats were used in this study. Rats were divided into six groups (8 rats each):

Group I (control group): Each rat received a single intratracheal injection of 0.1 ml PBS and received 1.5 ml DW and 1ml of PBS daily for 14 days orally by intragastric gavage tube.

Group II (BLM group): Each rat was injected intratracheally with a single dose of 0.1 ml bleomycin dissolved in sterile PBS.

Group III (BLM and Green tea group): Each rat was injected intratracheally with a single dose of 0.1 ml bleomycin dissolved in sterile PBS. From the first day of injection, each rat received 1.5 ml green tea extract dissolved in DW daily for 14 days orally by intragastric gavage tube.

Group IV (BLM and met group): Each rat was injected intratracheally with a single dose of 0.1ml dissolved bleomycin in sterile PBS. From the first day of injection, each rat received 1ml metformin dissolved in PBS daily for 14 days orally by intragastric gavage tube

Group V (green tea group): Each rat received 1.5 ml green tea extract dissolved in DW daily for 14 days orally by intragastric gavage tube.

Group VI (Metformin group): Each rat received 1 ml metformin dissolved in PBS daily for 14 day orally by intragastric gavage tube.

At the end of experimental period (14 days), all rats were sacrificed and the lung was resected from which sections were prepared and subjected to the following:

- 1) Hematoxylin and Eosin stain.
- 2) Mallory's trichrome stain.
- 3) Immunohistochemical staining for α -SMA.

Morphometric study and statistical analysis were performed for area% of collagen fibers distribution and of α -SMA immunoreactivity.

Group I (control group): showed normal spongy architecture of the lung, formed of many bronchi, bronchioles, alveolar ducts, sacs, and many alveoli. Bronchi were lined by pseudostratified columnar ciliated epithelium with goblet cells. The bronchioles were lined by simple columnar epithelium. The alveolar ducts and alveoli were lined with a simple squamous epithelium. The alveolar epithelium was formed of both type I and type II pneumocytes. Thin interalveolar septa were observed between the alveoli.

There was minimal collagen fibers deposition in the interalveolar septa, in the adventitia of blood vessels and in the outer connective tissue layer of bronchioles.

Immunoreaction for α -SMA was detected in the smooth muscle of the bronchioles, blood vessels and in knobs of alveolar ducts and negative reaction in the lining cells of the alveoli.

Group II (BLM group): showed extensive alveolar damage with inter- alveolar septal thickening and severe inflammatory cell infiltration, alveolar exudate, diffuse hemorrhagic area, fibrous areas, collapsed alveolar spaces, congested blood vessels and increase in the number of type II pneumocytes. Inflammatory cells were also found around small

airways and blood vessels. The lining epithelium of the bronchioles showed features of apoptosis. The smooth muscle layer of the bronchioles and blood vessels were thickened.

There was an obvious increase in collagen fibers deposition in the interalveolar septa, the adventitia of blood vessels and in the outer connective tissue layer of bronchioles.

There was statistically significant increase in area % of collagen compared to control group.

There was statistically significant increase in the area % of α -SMA compared to control group.

Group III (BLM and Green tea group): showed normal lung architecture with apparently thin interalveolar septa, few cellular infiltrations, normal bronchiolar structure, rarely observed interstitial hemorrhage and the blood vessels appear less congested.

There was a statistically significant decrease in area % of collagen compared to BLM group.

There was statistically significant decrease in the area % of α -SMA compared to BLM group.

Group IV (BLM and metformin group): showed normal lung architecture with thin interalveolar septa, few cellular infiltration, normal bronchiolar structure. The interstitial hemorrhage was still observed.

There was a statistically significant decrease in area % of collagen compared to BLM group.

There was statistically significant decrease in the area % of α -SMA compared to BLM group.

Both **group V and group VI** showed a picture more or less similar to that of the control.

The collagen area % was statistically non-significant difference compared to control group.

There was statistically non-significant difference in the area % of α -SMA compared to control group.

Conclusion

Bleomycin has profound cytotoxic effects on lung tissue. Green tea as a natural antioxidant with anti-inflammatory and antifibrotic effects, improved bleomycin induced lung injury. Metformin as an oral antidiabetic drug with anti-inflammatory and anti-tissue remodeling effects, had also the same effect as green tea in the protection against bleomycin induced lung injury except for minimal amount of hemorrhage and congested blood vessels.

Recommendation

- 1) Further experimental work should be done to clarify the safety of the long use of green tea and metformin on human without any side effects.
- 2) Further research is required to elaborate the precise mechanisms behind the curative effects of green tea and metformin.
- 3) Bleomycin shouldn't be used in patients suffering from interstitial lung diseases.
- 4) Further experimental work should be done to elaborate of the precise mechanisms behind the toxic effects of bleomycin.