Effect of Green Tea Extract on Experimentally Induced Lung Fibrosis in Adult Male Albino Rat

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Abstract: Background: Bleomycin is an antineoplastic agent used in the chemotherapy of different types of cancer. Its toxic effect has been utilized advantageously in experimental models to cause lung injury leading to oxidant induced inflammatory and fibrotic lesions. **Objectives:** The aim of this study was to determine the effect of green tea extract on the bleomycin induced lung fibrosis in adult male albino rats. **Methods:** This study was carried out on thirty male adult albino rats. The animals were divided into four groups, control group (I) consisted of ten rats divided into two subgroups five rats for each group. Green tea extract group (II) consisted of five rats. Lung fibrosis induction group (III) by bleomycin was consisted of five rats. Bleomycin and green tea extract group (IV) consisted of ten rats divided into two subgroups five rats for each one, subgroup IVa received green tea extract two weeks before bleomycin induction and was continued with bleomycin injection for four weeks to identify its protective role, subgroup IVb received green tea extract with the start of bleomycin injection for four weeks and continued addition two weeks to identify its therapeutic role. Rats were subjected to histological, immunohistochemical, morphometrical, genetical and statistical analysis. **Results:** Lung fibrosis showed marked accumulation of inflammatory cells, degeneration of lung alveolia, bronchiole and pulmonary arteriole with wide area of hemorrhage, hemosiderosis, excess collagen deposition. Immunohistochemical study showed, strong positive immunoreaction to inducible nitric oxide synthase and tumor necrosis factor alpha. Lung fibrosis was greatly reversed by addition of green tea extract. **Conclusion:** Lung fibrosis model greatly improved by addition of green tea extract, more prominent in its protective role. **Recommendations:** This results open the way to important applications in humans, where it could be recommended that patients receiving bleomycin or human being highly exposure to air pollutions should take green tea extract which is orally safe and inexpensive or at least drink green tea daily in a regular manner as a life style in order to reduce the affection of the lung with inflammation and fibrosis.

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Keywords: Department of anatomy and embryology, bleomycin, green tea extract, fibrosis, lung.

1. Introduction:

Pulmonary fibrosis is a disorder characterized by complex inflammatory processes that result in excessive fibroblast proliferation and progressive deposition of connective tissue in the pulmonary parenchyma **(Crestani *et al.*, 2007).** Bleomycin is an antineoplastic, Its toxic effect has been utilized advantageously in experimental models to cause lung injury leading to oxidant induced inflammatory and fibrotic lesions in the lung interstitium of various animal species **(Goto *et al.*, 2004).** This animal model of pulmonary fibrosis resembles that seen in humans and it is useful to assess the effects of potential therapeutic agents including antioxidants **(Serrano-Mollar *et al.*, 2003).** In recent years, a large effort has been taken to explore the possibility of using natural resources to delay progression of fibrosis. A great number of medicinal plants and their formulations are reported to possess antioxidant properties **(Gupta *et al.*, 2009).** Green tea extract contains wide-ranged anti-inflammatory characteristics, so it may be helpful in treating chronic inflammatory states **(Johnson and Williamson, 2003).** This work aimed at studying the effect of green tea extract on experimentally induced lung fibrosis by bleomycin administration in adult male albino rats.

2. Material and Methods:

Chemicals:

Bleomycin: Was imported from Naprod Life Sciences Pvt. Ltd, India. Supplied in the form of powder, vial of 15 mg and was dissolved in normal saline**.**

Green tea extract: Was obtained as tablets 200 mg from EL Obour For Modern company**.**

Inducible nitric oxide synthase: Iwas obtained from LabVision Corporation as liquid.

Tumor necrosis factor alpha: was obtained from LabVision Corporation as liquid. Both markers are rabbit polycolonal antibodies (IgG).

Animal protocol and experimental design:

Thirty male adult albino rats were obtained from Helwan Animal House. Their weight range from 150-200 gram. The animals were housed in metallic

cages, five rats in each cage, kept on standard diet in a healthy conditions during the whole experimental period. Guidelines for the ethical care and treatment of animals were followed. Animals were divided into Four groups:-

Group I (Control Group): Consisted of ten rats. This was subdivided into:

- Subgroup Ia: Consisted of five rats. They were fed on normal diet and kept without any treatment all over experimental period.
- Subgroup Ib: Consisted of five rats. They were received normal saline solution 0.9% "solvent of bleomycin" at the dose 3 ml/ rat intraperitonally three times a week for six weeks **(El-drieny *et al.*, 2009).**

Group II (Green tea extract group): Consisted of five rats. They were received green tea extract at a dose of (150mg/kg/day) orally by oral gavages **(Hamdy *et al.*, 2012**) dissolved in 3ml distilled water **(Liang *et al.*, 2010)** daily for six weeks.

Group III (Lung fibrosis induction group): Consisted of five rats. They were received bleomycin (15mg/kg/day) administrated intraperitoneally three times a week for four weeks **(Daba *et al.*, 2002) and (El–Medany *et al.*, 2005).**

Group IV (Bleomycin and green tea extract group): Consisted of ten rats. They were subdivided into:

- Subgroup IVa: Consisted of five rats. They were received green tea extract that administrated daily for two weeks then continued with bleomycin injection for four weeks **(Daba *et al.*, 2002) and (El–Medany *et al.*, 2005).**
- Subgroup IVb: Consisted of five rats. They were received green tea extract with bleomycin both for a total period of four weeks **(Daba *et al.*, 2002) and (El–Medany *et al.*, 2005).** Then continued with green tea extract daily for addition two weeks.

Bleomycin and green tea extract were administrated with the same above mentioned doses and routes of administration.

Histological and immunohistochemical studies:

According to above mentioned periods, rats were anaesthetized lightly by diethyl ether inhalation then sacrificed, thorax was opened. Lung was injected by neutral formol except one lung from each group as subjected to genetic study by agarose gel electrophoresis then other lung were washed by saline, injected by neutral formol divided into 2 equal parts, were subjected to different fixative according to type of study, one part saved in 10% neutral formol solution for 24 hours for histological study and other one was saved in bouin's solution for 24 hours for immunohistochemical study. Paraffin blocks were prepared and stained with H&E and Mallory's trichrome stains. Immunohistochemical study was performed using:

- Iducible nitric oxide synthase (iNOS) for detection of oxidative stress.
- Tumor necrosis factor alpha (TNF **α**) for detection of proinflammatory cytokines.

Genetic study: (DNA analytic study by agarose gel electrophoresis) The various fragments of DNA can be easily separated by using agarose gel electrophoresis by applying an electric field across the gel. DNA which is negatively charged at neutral pH, migrates towards the anode. The rate at which the fragments migrate through the gel is a function of their lengths, as small fragments moving much faster than larger fragments the relationship between the size of DNA fragment and the distance it migrates in the gel is logarithmic. Staining of the gel with ethediumbromid dye, that bind to DNA, generates a series of bands, each corresponding to the fragments whose molecular weight can be established by calibration with DNA molecule of known weight **(Southern, 1979)**.The gel electrophoretic results were analysed by gel pro-computer program.

Morphometric study and Statistical analysis:

Five different Mallory's trichrome stained sections from different rats were examined in each group to measure the percentage of surface area of collagen fibers in interalveolar septa, perivascular areas and peribronchiolar areas. Five different iNOS and other five TNF α stained sections from different rats were examined in each group to measure percentage of positive immunoreactivity cells count. This was done in Anatomy and Embryology department, Faculty of Medicine Menoufia university. Data were obtained using Lecia Qwin 500 image analyzer computer system. Statistical analysis was performed for the morphometrical results of the percentage of surface area of collagen fibers deposition in the interalveolar, peribronchiolar and perivascular area and percentage of iNOS, TNF α immunoreactivity positive cells in the lung induction group (group III) were compared with those in the other groups using the ANOVA test. The data was collected and tabulated.

3. Results:

There was no significant difference between Group I (control) and Group II (Green tea extract group) rats in all the outcomes at each time point used in the study; therefore, these two groups were pooled in one group.

Histological and immunohistochemical results:

Group I (control group)

H&E stained Lung sections showed numerous alveoli with thin alveolar walls, separated by thin interalveolar septa. The bronchioles were seen as small tube with folded mucosa lined by simple

cuboidal epithelium resting on a thin basal lamina surrounded by a smooth muscle layer. The interstitium of the lung contained branches of pulmonary artery with intact lining endothelium**.** Both bronchiole and pulmonary arteriole were surrounded by few lymphocytic cells **(Figs 1-3).** Mallory's trichrome of lung sections revealed minimal amount of collagen fibers appears in alveolar walls, perivascular and peribronchiolar areas **(Figs 4, 5).** Immunostaining of lung sections revealed few number of positive immunoreactivity cells of iNOS and TNF α **(Figs 6, 7).**

Group III (Lung fibrosis induction group):

H&E stained Lung sections showed loss of the normal alveolar architecture, massive inflammatory cellular infiltration resulted in thickening of interalveolar septa with narrowing of some alveolar spaces, obliteration of other alveoli and compensatory dilatation of adjacent ones with emphysematous changes**.** Extravastion of some red blood cells in the alveolar spaces and hemosiderosis were noted**.** The bronchiolar wall appeared markedly thickened as thickening of its muscular layer and heavily peribronchiolar inflammatory cellular infiltration. Some bronchioles showed partial destruction of their lining epithelium with appearance of cellular depris inside their lumen**.** The wall of pulmonary arteriolar appeared markedly thickened as thickening of its tunica media and massive perivascular inflammatory cellular infiltration with destruction of its lining endothelium**.** Some arterioles appeared congested **(Figs 8-14).** Mallory's trichrome stain of lung sections revealed an increase in collagen fibers of the interalveolar septa, peribronchiolar and perivascular areas as compared to control group **(Figs 15-17).** Immuohistochemical study of lung sections revealed many immunoreactivity cells of iNOS and TNF α **(Figs 18, 19).**

Group IV (Bleomycin and green tea extract group):

Great improvement occurred in both subgroups as less disturbance of normal alveolar architecture, but improvement in subgroup IVa is more prominent than subgroup IVb.

a-Subgroup IVa:

H&E stained Lung sections showed decreased inflammatory cellular infiltration, decreased thickness of interalveolar septa**.** The peribronchiolar cellular infiltration was decreased. Bronchiolar epithelium and pulmonary arteriole lining endothelium appeared more or less intact**.** Marked decreased in perivascular cellular infiltration. Interstitial hemorrhage was rarely observed **(Figs 20-22).** Mallory's trichrome stained revealed decrease in collagen fibers of the interalveolar septa, peribronchiolar and perivascular areas **(Figs 23, 24).** Immunohistochemical study revealed few number of immunoreactivity cells of iNOS and TNF α **(Figs 25, 26).**

b-Subgroup IVb:

H&E stained lung sections showed decreased inflammatory cellular infiltration, decreased thickness of interalveolar septa and other areas showed increased inflammatory cellular infiltration and increased thickness of interalveolar septa in the same lung section**.** The peribronchiolar inflammatory cellular infiltration was decreased and bronchiolar epithelium appeared more or less intact**.** The perivascular inflammatory cellular infiltration was decreased and pulmonary arterioles appeared less congested**.** Interstitial hemorrhage and hemosiderosis granules were less observed **(Figs 27-29).**

Mallory's trichrome revealed decreased in collagen fibers in interalveolar septa, peribronchiolar and perivascular areas **(Figs 30-32).** Immunohistochemical study revealed some positive immunoreactivity cells of iNOS and TNF α **(Figs 33, 34).**

Genetic result:

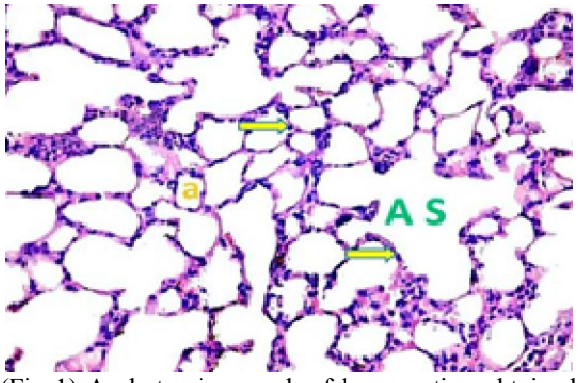
As molecular detection of DNA fragmentation by agarose gel electrophoresis (**Fig 35).**

Group I, there was no apoptotic bands could be detected in lane 1.

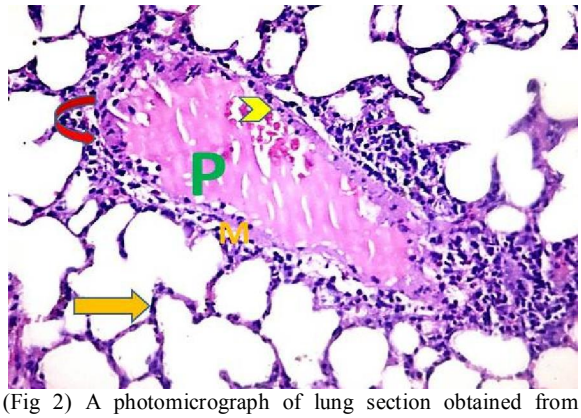
Group II, there was no apoptotic bands could be detected in lane 2.

Group III, apoptotic bands in the form of a ladder–like DNA fragmentation pattern which was a characteristic of apoptosis, were detected in lane 3.

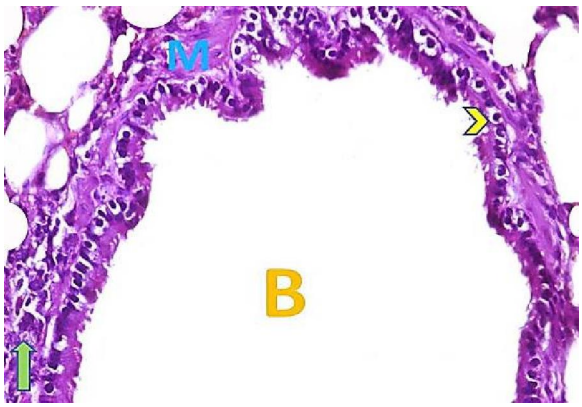
Group IV divided into, subgroup IVa: apoptotic band could be detected in lane 4, lesser than group III. Subgroup IVb: apoptotic band could be detected in lane 5, lesser than group III and more than subgroup IVa.



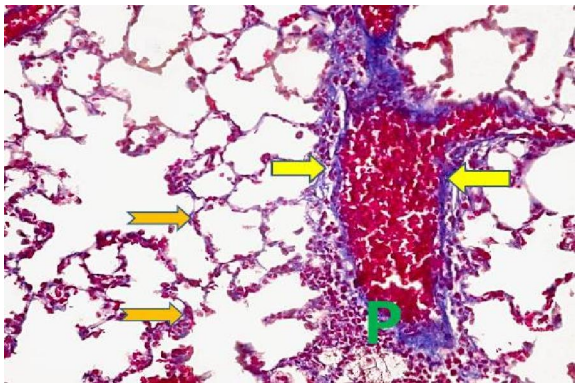
(Fig 1) A photomicrograph of lung section obtained from group I (control group) showing alveolar sac **(A S)** and numerous alveoli **(a)** with normal thickness of interalveolar septum **(arrow). (H&Ex400).**



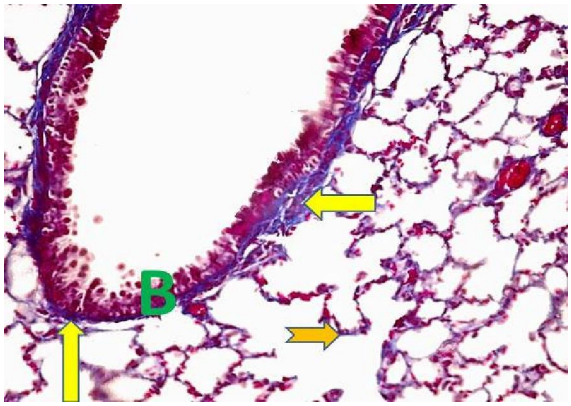
(Fig 2) A photomicrograph of lung section obtained from group I (control group) showing pulmonary arteriole **(P)** with intact lining endothelium **(arrow head) and** tunica media **(M)** with little perivascular lymphocytic infiltration **(curved arrow).** Noticed thin interalveolar septum **(arrow). (H&Ex400).**



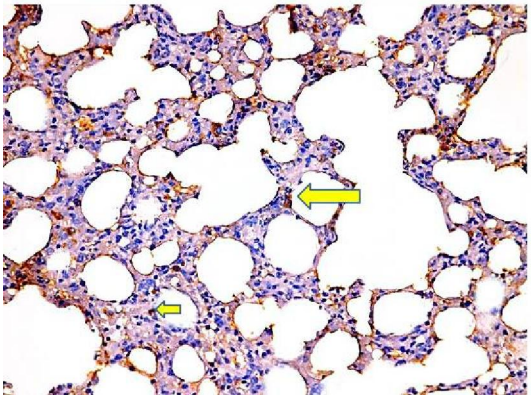
(Fig 3) A photomicrograph of a lung section obtained from group I (control group) showing bronchiole **(B)** with intact lining ciliated cubidal epithelium **(arrow head)** and layer of smooth muscle fibers **(M).** Noticed little peribronchiolar lymphocytic cellular infiltration **(arrow).**
(H&Ex400).



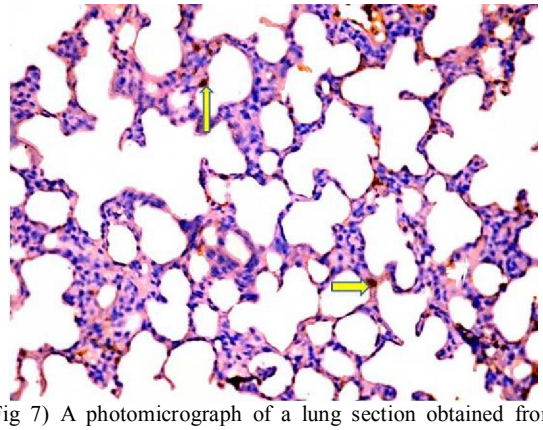
(Fig 4) A photomicrograph of a lung section obtained from group I (control group) showing minimal collagen fibers deposition **(arrow)** in the perivascular area **(p)** and interalveolar septum **(notched arrow).**
(Mallory's trichrome, x400).



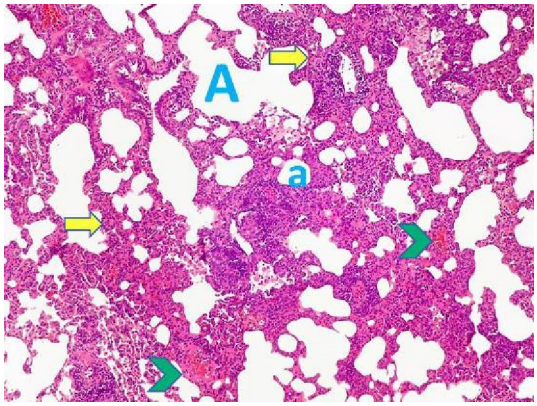
(Fig 5) A photomicrograph of a lung section obtained from group I (control lung) showing minimal collagen fibers deposition **(arrow)** in the peribronchiolar area **(B)** and interalveolar septum **(notched arrow).**
(Mallory's trichrome, x400).



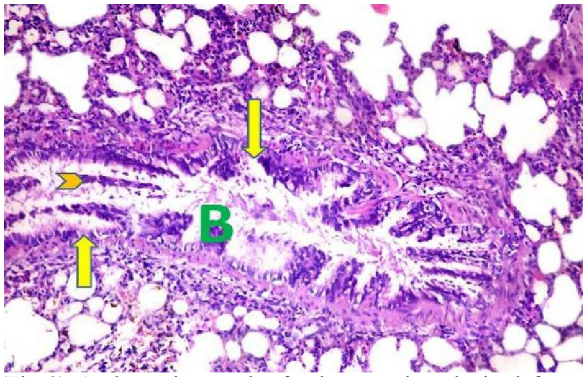
(Fig 6) A photomicrograph of a lung section obtained from group I (control group) showing few number of positive immunoreactivity cells of inducible nitric oxide synthase (iNOS) in the lung parenchyma **(arrow).**
(iNOS., x400).



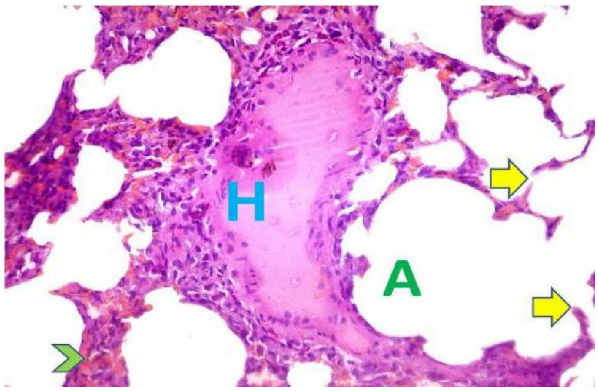
(Fig 7) A photomicrograph of a lung section obtained from group I (control group) showing few number of positive immunoreactivity cells of tumor necrosis factor alpha (TNFα) in the lung parenchyma **(arrow).**
(TNFα., x400).



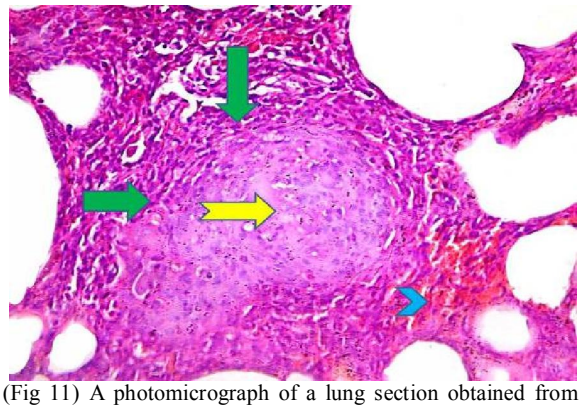
(Fig 8) A photomicrograph of a lung section obtained from group III (Lung fibrosis induction group) showing thickening of the interalveolar septa **(arrow)** as accumulation of inflammatory cells resulting in narrowing of some alveolar spaces **(a)** and compensatory dilatation of adjacents ones **(A).** Noticed area of hemorrhage **(arrow head).**
(Hx&E., x200).



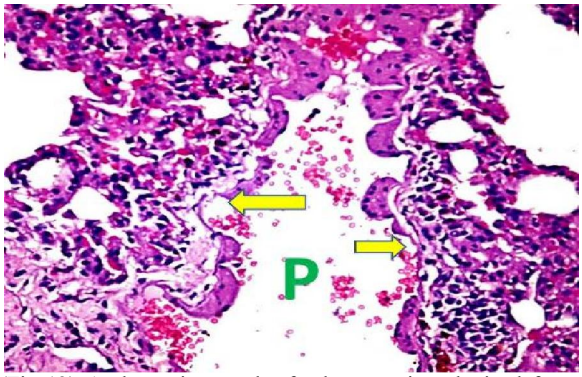
(Fig 9) A photomicrograph of a lung section obtained from group III (Lung fibrosis induction group) showing bronchiole **(B)** with marked desqumation of its lining epithelial **(arrow)** and cellular depris in its lumen **(arrow head).**
(Hx&E., x200).



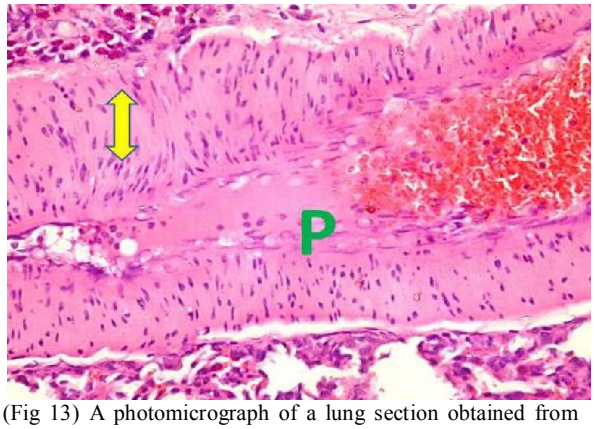
(Fig 10) A photomicrograph of a lung section obtained from group III (Lung fibrosis induction group) showing rupture of interalveolar septa **(arrow)** leading to large irregular air space **(A).** Noticed area of interstitial hyaline degeneration **(H)** and hemosiderosis **(arrow head).**
(Hx&E., x200).



(Fig 11) A photomicrograph of a lung section obtained from group III (Lung fibrosis induction group) showing focal aggregation of inflammatory cells **(arrow)** with central degenerative change **(notched arrow).** Noticed area of hemorrhage **(arrow head).**
(Hx&E., x400).



(Fig 12) A photomicrograph of a lung section obtained from group III **(**Lung fibrosis induction group) showing pulmonary arteriole **(P)** with massive destruction of its lining endothelium **(arrow).**
(Hx&E., x400).



(Fig 13) A photomicrograph of a lung section obtained from group III (Lung fibrosis induction group) showing pulmonary arteriole **(P)** with marked thickness of tunica media **(douple head arrow)**
(Hx&E., x400).

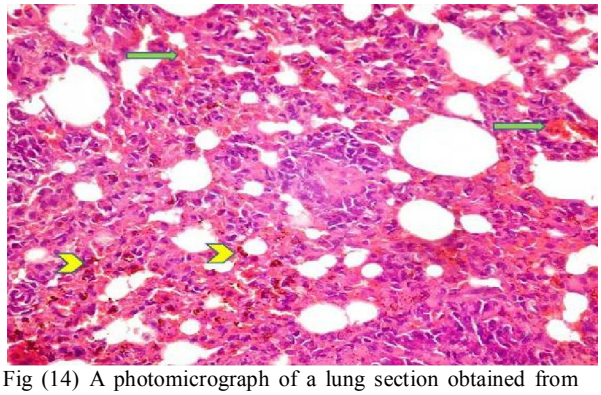
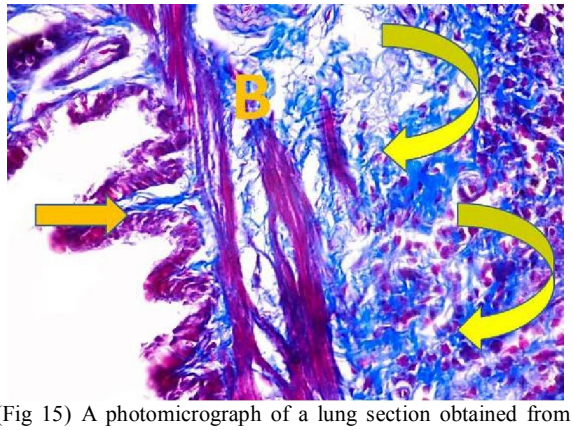
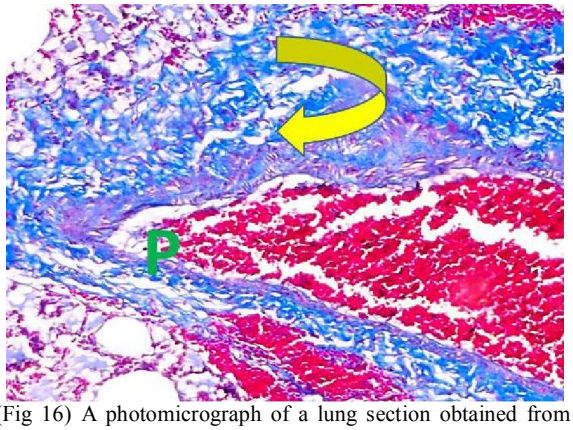


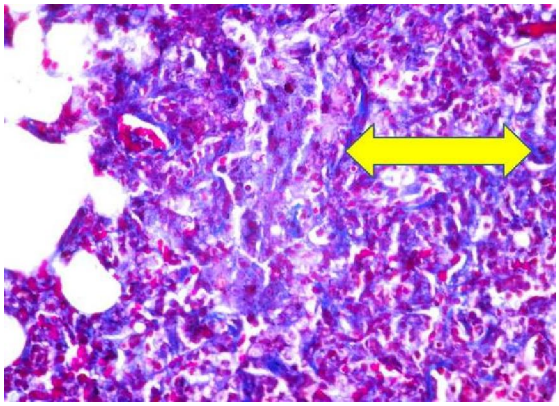
Fig (14) A photomicrograph of a lung section obtained from group III (Lung fibrosis induction group) showing diffuse interalveolar hemorrhage **(arrow)** and hemosiderosis granules in interstitial pulmonary paranchyma **(arrow head).**
(Hx&E., x400).



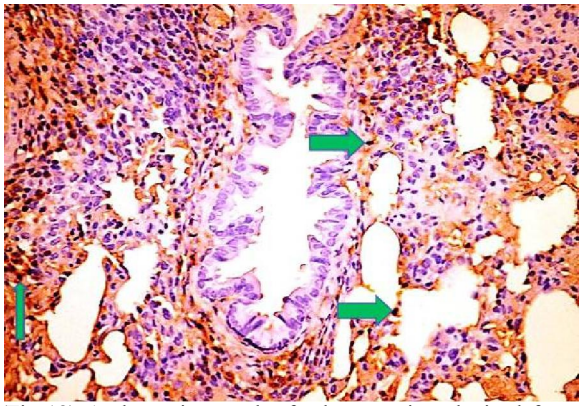
(Fig 15) A photomicrograph of a lung section obtained from group III (Lung fibrosis induction group) showing excess collagen fibers deposition **(curved arrow)** in the peribronchiolar area **(B).** Noticed subepithelium collagen fibers deposition **(arrow).**
(Mallory's trichrome, x400).



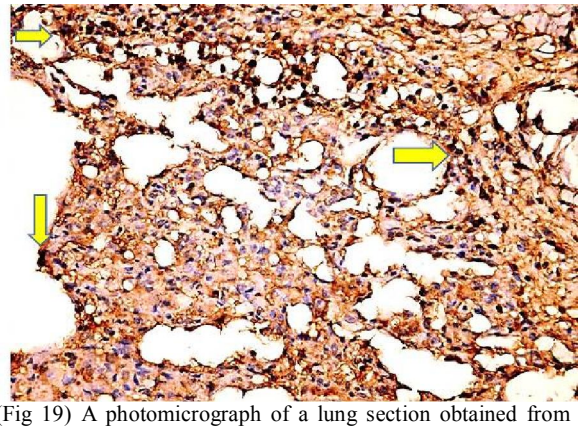
(Fig 16) A photomicrograph of a lung section obtained from group III (Lung fibrosis induction group) showing excess collagen fibers deposition **(curved arrow)** in the perivascular area **(P).**
(Mallory's trichrome, x400).



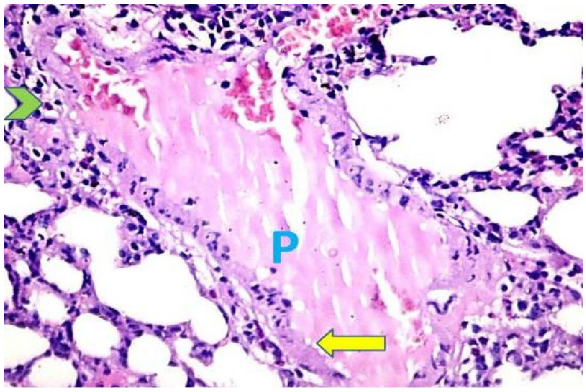
(Fig 17) A photomicrograph of a lung section obtained from group III showing excess collagen fibers deposition **(douple head arrow)** in interalveolar area.
(Mallory's trichrome, x400).



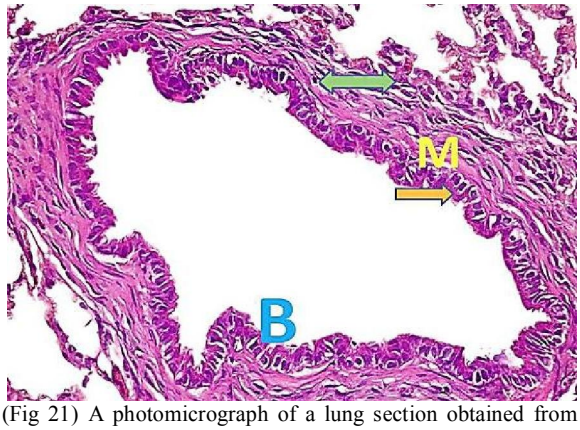
(Fig 18) A photomicrograph of a lung section obtained from group III (lung fibrosis induction group) showing many positive immunoreactivity cells of inducible nitric oxide synthase (iNOS) in the lung paranchyma **(arrow)**
(INOS., x400).



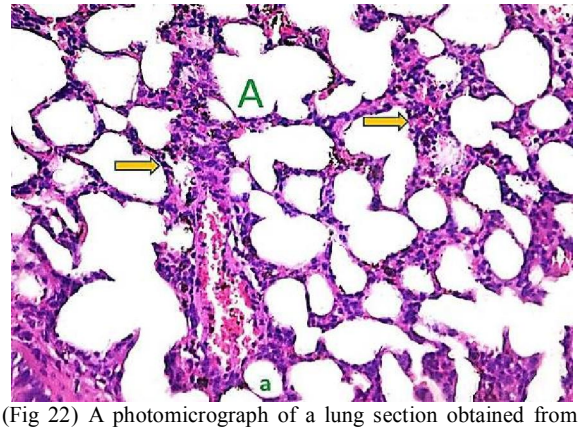
(Fig 19) A photomicrograph of a lung section obtained from group III (lung fibrosis induction group) showing many positive immunoreactivity cells of tumor necrosis factor alpha (TNFα) in the lung paranchyma **(arrow) (TNFα., x400).**



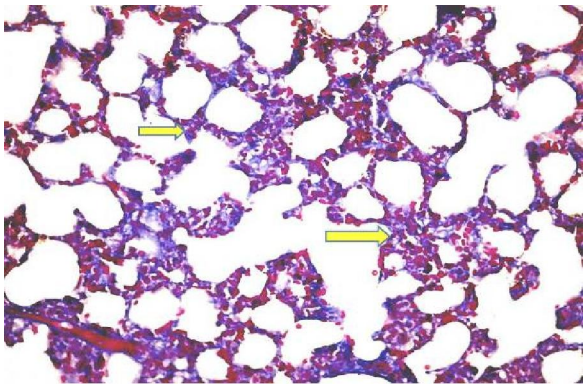
(Fig 20) A photomicrograph of a lung section obtained from subgroup IVa (bleomycin and green tea extract subgroupa) showing pulmonary arteriole **(P)** with more or less intact of its lining endothelium **(arrow)** and less perivascular inflammatory cellular infiltration **(arrow head)** as compared to group III. **(Hx&E., x400).**



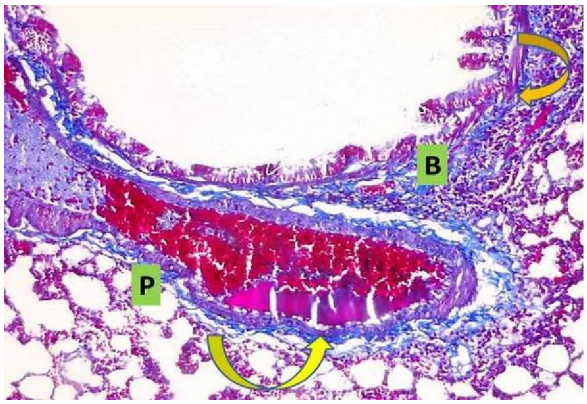
(Fig 21) A photomicrograph of a lung section obtained from subgroup IVa (bleomycin and green tea extract subgroupa) showing bronchiole **(B)** with less disturbance of lining ciliated cuboidal epithelium **(arrow),** less thickness of the muscular layer in its wall **(M)** also less peribronchiolar inflammatory cells infiltration **(douple head arrow)** as compared to group III. **(Hx&E., x400).**



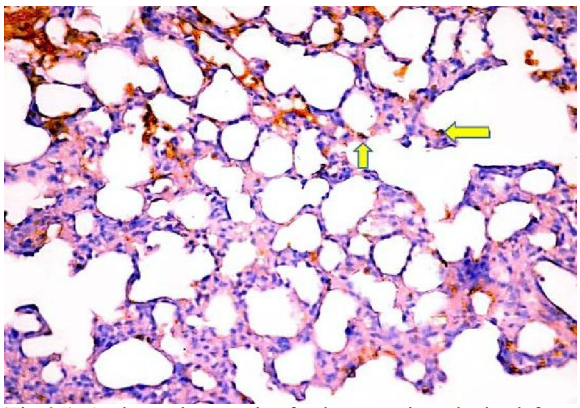
(Fig 22) A photomicrograph of a lung section obtained from subgroup IVa (bleomycin and green tea extract subgroupa) showing less thickning of interalveolar septum **(arrow)** as compared to group III. Noticed alveolar sac **(A)** and alveola **(a). (Hx&E., x400).**



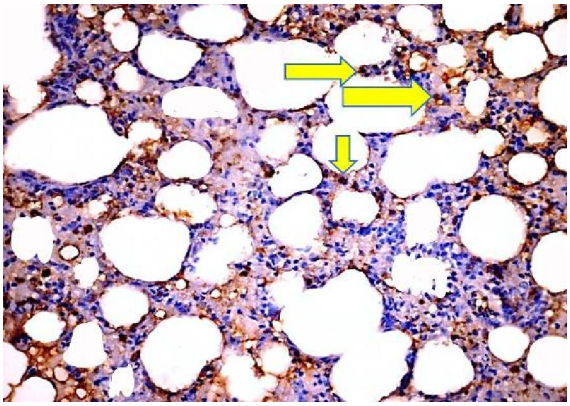
(Fig 23) A photomicrograph of a lung section obtained from subgroup IVa (bleomycin and green tea extract subgroupa) showing less collagen fibers deposition **(arrow)** in the interalveolar septum as compared to group III **(Mallory's trichrome, x400).**



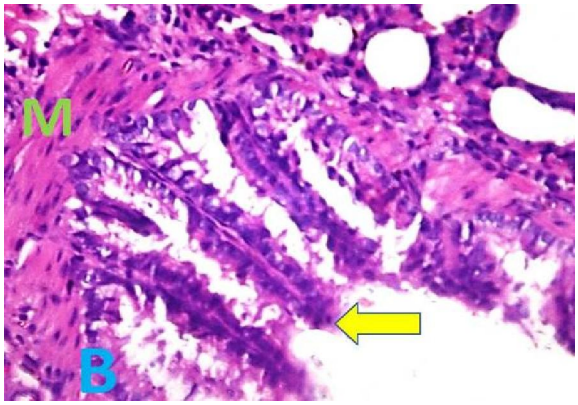
(Fig 24) A photomicrograph of a lung section obtained from subgroup IVa (bleomycin and green tea extract subgroupa) showing less collagen fibers deposition **(curved arrow)** in the peribronchiolar area **(B)** and perivascular area **(P)** as compared to group III. **(Mallory's trichrome, x400)**.



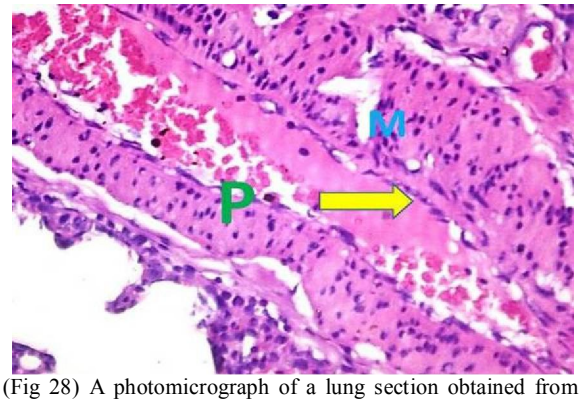
(Fig 25) A photomicrograph of a lung section obtained from subgroup IVa (bleomycin and green tea extract subgroupa) showing few number of positive immune reaction to inducible nitric oxide synthase (iNOS) in the lung paranchyma **(arrow). (INOS., x400).**



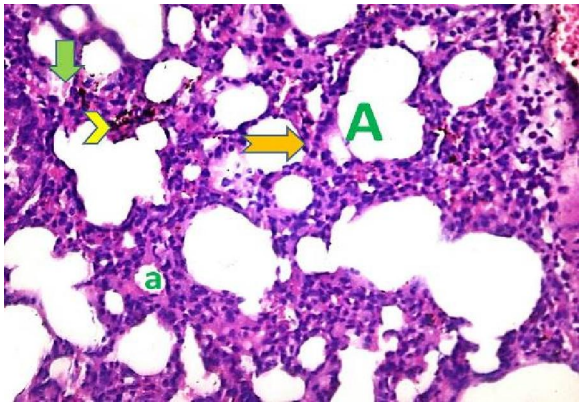
(Fig 26) A photomicrograph of a lung section obtained from subgroup IVa (bleomycin and green tea extract subgroupa) showing few number of positive immunoreactivity cells of tumor necrosis factor alpha (TNFα) **(arrow).**
(TNFα., x400).



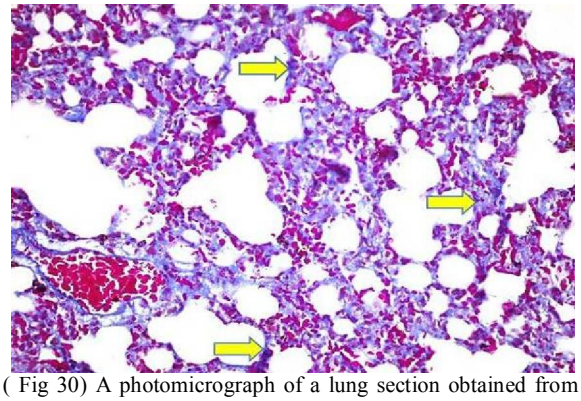
(Fig 27) A photomicrograph of a lung section obtained from subgroup IVb (bleomycin and green tea extract subgroupb) showing bronchiole **(B)** with less destruction of its linning epithelium **(arrow),** less thickness of its muscular layer **(M)** as compared to group III but more than in subgroup Iva. **(Hx&E., x400).**



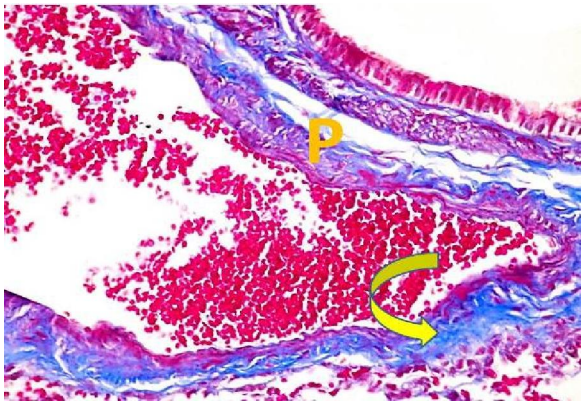
(Fig 28) A photomicrograph of a lung section obtained from subgroup IVb (bleomycin and green tea extract subgroupb) showing less congestion of pulmonary arteriole **(P),** less thickness of its tunica media **(M)** as compared to group III but more than subgroup IVa. Noticed more or less intact of its lining endothelium **(arrow).**
(Hx&E., x400).



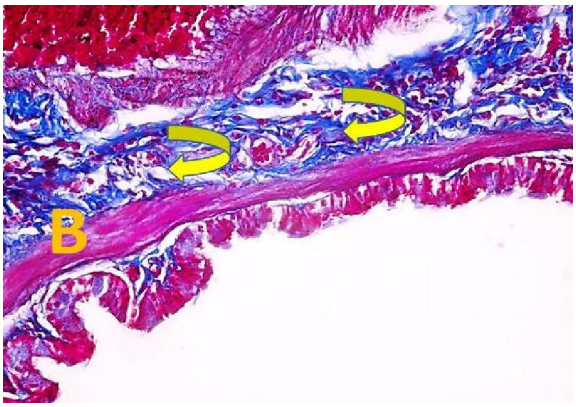
(Fig 29) A photomicrograph of a lung section obtained from subgroup IVb (bleomycin and green tea extract subgroup IVb) showing less thickening of interalveolar septum **(notched arrow)**, small irregular air spaces **(a)** with compansatory diltation of adjacent one **(A)** as compared to group III but more than subgroup IVa. Noticed hemosidren granules **(arrow head)** and hemorrhage **(arrow).**
(Hx&E., x400).



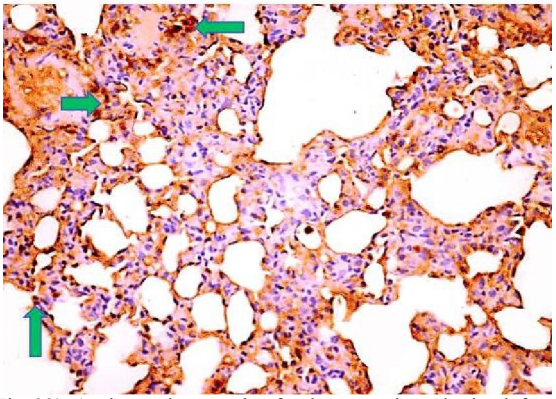
(Fig 30) A photomicrograph of a lung section obtained from subgroup IVb (bleomycin and green tea extract subgroup b) showing less collagen fibers deposition **(arrow)** in the interalveolar septa as compared to group III but more than subgroup Iva.
(Mallory's trichrome, x400).



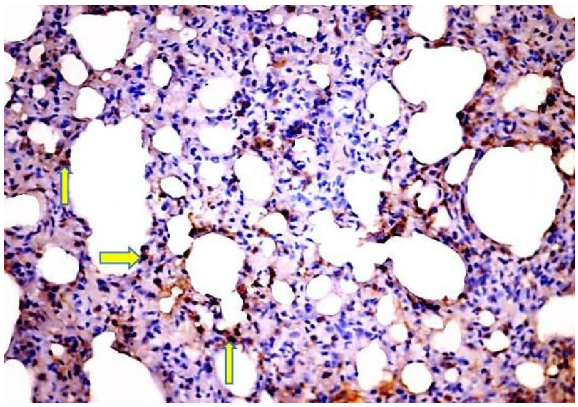
(Fig 31) A photomicrograph of a lung section obtained from subgroup IVb (bleomycin and green tea extract subgroupb) showing less collagen fibers deposition **(curved arrow)** in the perivascular area **(P)** than in group III but more than subgroup Iva**. (Mallory's trichrome, x400)**.



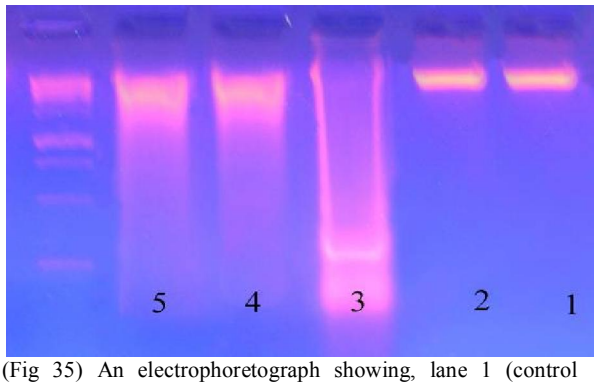
(Fig 32) A photomicrograph of a lung section obtained from subgroup IVb (bleomycin and green tea extract subgroupb) showing less collagen fibers deposition **(curved arrow)** in the perbronchiolar area **(B)** as compared to group III but more than subgroup Iva.
(Mallory's trichrome, x400).



(Fig 33) A photomicrograph of a lung section obtained from subgroup IVb (bleomycin and green tea extract subgroupb) showing some positive immunoreactivity cells of inducible nitric oxide synthase (iNOS) in the lung paranchyma **(arrow).**
(iNOS., x400).



(Fig 34) A photomicrograph of a lung section obtained from subgroup IVb (bleomycin and green tea extract subgroup b) showing some positive immunoreactivity cells to tumor necrosis factor alpha (TNFα) in the lung paranchyma **(arrow).**
(TNFα., x400).



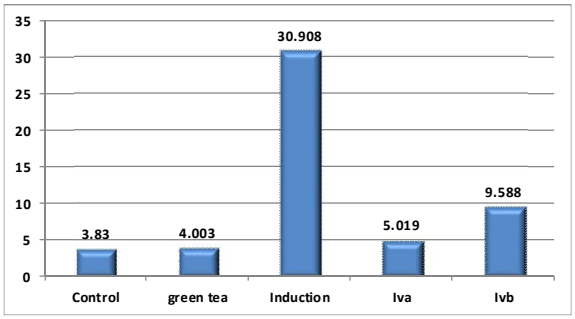
(Fig 35) An electrophoretograph showing, lane 1 (control group) showing no fragmentation of DNA, lane 2 (green tea extract group) showing no fragmentation of DNA, lane 3 (lung fibrosis inducted group) showing severe fragmentation of DNA, lane 4 (Group IVa) showing less fragmentation of DNA and lane 5 (Group IVb) showing less fragmentation of DNA.

Statistical results:

Regarding to mophometrical study, percentage of iNOS immunoreactivity positive cells count {Table (1) and Histogram (1)}.

Table (1): Regarding percentage of positive immunostaning cells count in iNOS stained lung section.

INOS	Mean	Std. Deviation	ANOVA	P value	Post hoc LCD	
Group1	3.830	.663	401.509	< 0.001	*P*1 < 0.001	HS
					P 2 > 0.05	NS
					P 3 < 0.001	HS
Group 11	4.003	.864			*P* 4 > 0.05	NS
					P 5 <0.001	HS
					P 6 < 0.001	HS
Group III	30.908	2.357			*P* 7< 0.001	HS
Group IV: SubgroupIva	5.0190	.951				
SubgroupIvb	9.588	.813				

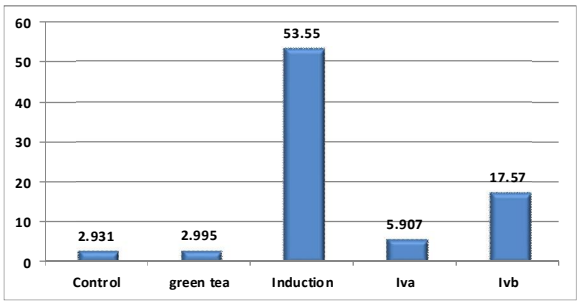


Histogram (1): Regarding percentage of positive cells count in iNOS immunostaning.

Regarding to mophometrical study, percentage of TNFα immunoreactivity positive cells count {Table (2) and Histogram (2)}.

Table (2): Regarding percentage of positive immunostaning cells count in TNFα stained lung section.

TNFα	Mean	Std. Deviation	ANOVA	P value	Post hoc LCD	
Group I	2.931	.529	1230.0	< 0.001	*P* 1 < 0.001	HS
Group II	2.995	.480			*P* 2 < 0.05	S
					P 3 < 0.001	HS
					P 4 > 0.05	NS
					P 5 <0.001	HS
Group III	53.55	1.366			*P* 6 < 0.001	HS
Group IV: SubgroupIva	5.907	.408			*P* 7< 0.001	HS
SubgroupIvb	17.57	2.621				

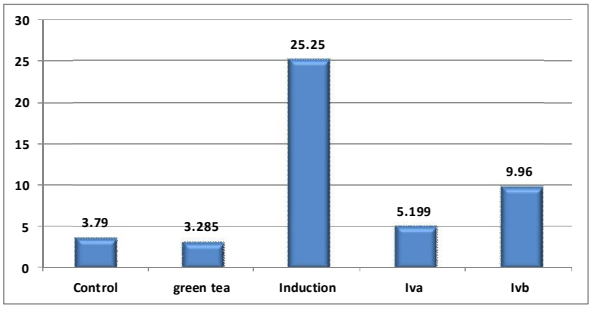


Histogram (2): Regarding percentage of positive cells count in TNFα immunostaning.

Regarding to mophometrical study, percentage of collagen fibers surface area in interalveolar septa {Table (3) and Histogram (3)}.

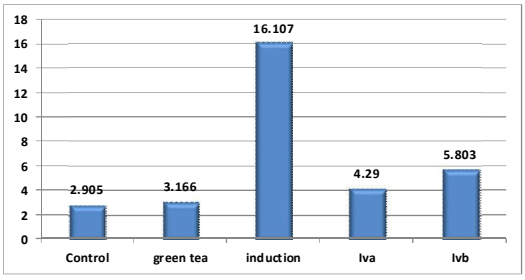
Table (3): Regarding percentage of collagen fibers surface area in interalveolar septa.

Alveolus	Mean	Std. Deviation	ANOVA	P value	Post hoc LCD	
Group 1	3.790	1.227	251.14	.< 0.001	*P* 1 < 0.001	HS
GroupII	3.285	.654			*P* 2 > 0.05	NS
					P 3 < 0.001	HS
					P 4 > 0.05	NS
					P 5 <0.001	HS
GroupIII	25.25	1.468			*P* 6 < 0.001	HS
GroupIV SugroupIva	5.199	1.001			*P* 7< 0.001	HS
SubgroupIvb	9.960	1.821				



Histogram (3): Regarding percentage of collagen fibers surface area in interalveolar septa.

Regarding to mophometrical study, percentage of collagen fibers surface area in pulmonary perivascular areas {Table (4) and Histogram (4)}.



Histogram (4): Regarding percentage of surface area of collagen fibers deposition in pulmonary perivascular areas.

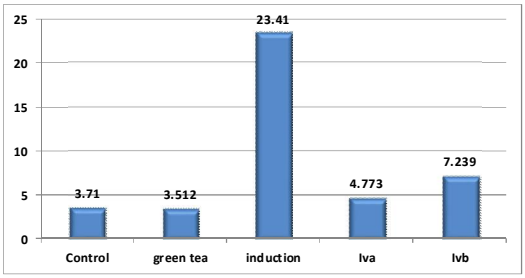
Table (4): Regarding percentage of surface area of collagen fibers deposition in pulmonary perivascular areas.

Arteriols	Mean	Std. Deviation	ANOVA	P value	Post hoc LCD	
GroupI	2.905	.469	189.51	< 0.001	*P* 1 < 0.001	HS
Group II	3.166	.463			*P* 2 < 0.05	S
					P 3 < 0.001	HS
					P 4 > 0.05	NS
Group III	16.107	1.454			*P* 5 <0.001	HS
Group IV SubgroupIva	4.290	.591			*P* 6 >0.05	S
SubgroupIvb	5.803	1.054			*P* 7> 0.05	NS

Regarding to mophometrical study, percentage of collagen fibers surface area in peribronchiolar areas {Table (5) and Histogram (5)}.

Table (5): Regarding percentage of surface area of collagen fibers deposition in peribronchiolar areas.

Bronchioles	Mean	Std. Deviation	ANOVA	P value	Post hoc LCD	
Group 1	3.710	.569	206.248	< 0.001	*P* 1 < 0.001	HS
Group II	3.512	.659			*P* 2 > 0.05	NS
					P3 < 0.001	HS
					P4 > 0.05	NS
Group III	23.410	2.383			P5 <0.001	HS
Group IV: SubgroupIva	4.773	1.210			P6 < 0.001	HS
SubgroupIvb	7.239	.864			P7< 0.05	S



Histogram (5): Regarding percentage of surface area of collagen fibers deposition in peribronchiolar areas.
P. value > 0.05 non significant (NS).
P. value < 0.05 significant (S).
P. value < 0.001 highly significant (HS).
P^1 between control and induction
P^2 between control and IVa
P^3 between control and IVb
P^4 between control and green tea
P^5 between induction and IVa
P^6 between induction and IVb
P^7 between IVa and IVb.

4.Discussion:

Several studies demonstrated that exposure to bleomycin increased the risk for pulmonary fibrosis and impaired lung function. On the other hand, green tea extract proved to has anti-inflammatory, anti-fibrotic and antioxidants effect on the lung. Thus, the present study was performed to induce rat lung fibrosis model and investigate the possible effective role of green tea extract.

In the present work, lung tissue was selected as the lung is a target organ for toxicity caused by a variety of xenthobiotics due to envitomental exposure to mineral dusts, airborne pollutants, cigarette smoke or pharmacological therapy with anticancer drugs that lead to inflammatory lung diseases and fibrosis as mentioned by **Venkatesan *et al.*, (2007).**

Bleomycin was chosen in this work as it is an antineoplastic agents by causing oxidant damage to DNA. This toxic effect has been utilized advantangeously in experimental models to cause lung injury leading to oxidant induced inflammatory and fibrotic lesions in the lung interstitium of various animal species as stated by **Goto *et al.*, (2004).**

In the present study we used green tea extract as antioxidants properites. As **Frie and Higdon, (2003)** stated that green tea extract has been reported to be useful as antioxidants.

The selection of the dose of bleomycin to induce lung fibrosis was based on previously published data of **Daba *et al.*, (2002)**, **El–Medany *et al.*, (2005) and Hegazy *et al.*, (2010)** who found that induction model of lung fibrosis treated with bleomycin (15 mg/kg, i.p.), three times a week for a total period of 4 weeks. As **Gado and Yassen (2012)** explained that this dose induced lung toxicity as indicated by a significant increased in the level of

lipid peroxide, significant depletion of the reduced glutathione in lung tissue and significant increased in the activity of antioxidant enzymes.

In the present study of lung fibrosis induction group showed by H&E study massive inflammatory cellular infiltration, thickening of interalveolar septa, areas of consolidation. Some specimens also showed ruptured interalveolar septa with the formation of large irregular air spaces. This was in agreement with **El–Medany *et al.*, (2005), Hegazy *et al.*, (2010) and Kumar *et al.*, (2011)** who reported that the lung fibrosis induction rats showed inflammatory cell infiltrated in the alveoli and interstitium, collapsed alveoli, marked thickening of the interalveolar septa.

The interstitial hemorrhage was detected. Hemorrhage was reported by **Goto *et al.*, (2004)** who explained the cause of intra-alveolar hemorrhage in rat pulmonary fibrosis is injury of the blood-air barrier allowing leakage of red blood cells in case of intra-alveolar space.

In current study of lung fibrosis induction rats noticed destruction of the lining epithelium of alveoli and bronchiole as well as destruction of endothelium lining of the pulmonary arteriole. The findings of the present study were in agreement with **Chen *et al.*, (2010)** who reported that the bleomycin instilled lung fibrosis showed sever epithelial degeneration and cellular breakdown were observed.

In this study, noticed marked thickening tunica media of pulmonary arteriole. The findings in agreement with **Ali and Mansour, (2011)** who found that increased wall thickness of pulmonary arteriole in lung fibrosis induction rat. The biochemical basis of this structural remodeling of pulmonary arterioles was explained by the study of **Cui *et al.*, (2008)** who noticed an increase in growth factors in arteriolar smooth muscle layer following bleomycin administration.

Also found in present study, marked thickening of bronchiolar wall. The finding in agreement with **Hegazy *et al.*, (2010)** who found that massive fibrotic changes and thickening of bronchiolar wall following induction of lung fibrosis.

Moreover in the present study, vacuolation of bronchiolar lining epithelium. This result in agreement with **Kim *et al.*, (2009)** who mentioned that the bronchiolar epithelial cells were swollen showing vacoular changes.

By Mallory's trichrome stained in the present work of lung fibrosis induction rats, excessive collagen deposition causing thickening of the interalveolar septa. Moreover, in this study also noticed collagen deposition was not limited to the lung parenchyma but also seen in the adventitia of the bronchioles and arterioles causing evident thickening in their walls. These results were in agreement with the previous findings reported by **El–Medany *et al.*, (2005)** who noticed that excessive amount of collagen deposited around the alveoli in the lungs of rats treated with bleomycin, Also, **Hemmati *et al,*. (2011)** mention that in lung fibrosis induction rats, there was increased peribronchial or perivascular fibrosis was seen in the most tissue section.

All these results were explained by **Barqaqli *et al.*, (2009)** who reported that oxidative stress with reactive oxygen species (ROS) plays an important role in the process of pulmonary fibrosis. And also supported by **Corrine and Oury, (2010)** who mentioned that the presence of oxidative stress in the lung can lead to increased inflammation and fibrosis**.**

In this study, lung fibrosis induction by bleomycin was studied immunhistochemical by iNOS demonstrated many immunoreactivity positive cells compared to control group. This finding was in agreement with **Kalayarasan *et al.*, (2008)** who mentioned that activation of inducible nitric oxide synthase in rat lung tissue of lung fibrosis induction group. **Mungrue *et al.*, (2002)** explained induction of the high-output iNOS usually occurs in an oxidative environment and thus high levels of nitric oxide have the opportunity to react with superoxide leading to peroxynitrite formation and cell toxicity.

This study also studied immunhistochemically by (TNFα) demonstrated that many immunoreactivity positive cells to TNF α compared to control group. The finding of the present study was in agreement with **Kalayarasan *et al.*, (2008)** who found that increased level of TNFα in rat lung tissue of bleomycin induced lung fibrosis group. **Muppidi *et al.*, (2004)** explained that TNF-α is a proinflammatory cytokine that plays a critical role in diverse cellular events. The binding of TNF-α to TNF-α receptors triggers a series of intracellular events that ultimately result in production of inflammatory cytokines.

In the present study, lung fibrosis induction by bleomycin were studied genetically, detected marked DNA fragmentation. The finding of the present study was in agreement with **Kumar *et al.*, (2011)** who mentioned that in bleomycin treated group, DNA smear showed the fragments compare to control group. These results were explained by **Atzori *et al.*, (2004)** who reported that the mechanism of bleomycin-induced pulmonary injury and fibrosis is known to generate reactive oxygen species upon binding to DNA and iron, which cause DNA damage**.**

In the current study, observed that pulmonary fibrosis induction rats which received green tea extract revealed less damage in most specimens. Decreased alveolar cell injury, inflammatory cellular infiltration, hemorrhage, areas of consolidation and collapse were much less encountered than in rats of

lung fibrosis. Also, the thickening of the interalveolar septa, bronchiolar and arteriolar walls were decreased. The findings of the present study were in agreement with **El-Sayed and Rizk, (2009).**

Also by Mallory's trichrome stain, detected greatly decreased in the level of collagen fibers deposition in pulmonary interstitium, perivascular and peribronchiolar areas. The findings of the present study were in agreement with **Kim *et al.*, (2006) and Hamdy *et al.*, (2012)** who mentioned that green tea extract groups showed few collagen deposition as compared to the lung fibrosis induction group.

In the present study of rats with lung fibrosis treated with green tea extract, by immunohistochemical study, noticed that few number of inducible nitric oxide synthase immunoreactivity cells as compared to induction group. These results were in agreement with the previous findings reported by **Hamady *et al.*, (2012)** who mentioned that treatment with green tea markedly normalized the elevated nitric oxide level. These results were in disagreement with the previous findings reported by **El-Sayed and Rizk, (2009)** who mentioned that green tea did not significantly lower elevated nitric oxide levels as compared to inducted group.

The previous results were explained by **Katiyar *et al.*, (1999)** who mentioned that green tea polyphenols inhibit the production of arachidonic acid metabolites such as pro-inflammatory prostaglandins and leukotrienes, resulting in a decreased inflammatory response. Also **Sriram *et al.*, (2008)** mentioned that green tea extract reduced free radical generation and oxidative stress during pulmonary fibrosis.

In the present study of rats treated by bleomycin and green tea extract, immunohistochemically noticed greatly decreased TNFα immunoreactivity as compared with induction group. These results were in agreement with **Sueoka *et al.*, (2001)** who mentioned that expressions of TNF-α was inhibited in the pulmonary fibrosis of mice after treated with green tea.

This result was explained by **Okabe *et al.*, (1999)** who mentioned that green tea extract has been found to modulate cytokine expression related to inflammation**.**

The present study demonstrated green tea extract improved DNA injury detected in the lung fibrosis inducted rats. These results were explained by **Tipoe *et al.*, (2007)** who mentioned that green tea extract can act as electron traps to scavenge free radicals, inhibit the formation of reactive oxygen spieces and reduce oxidative stress.

In contrast, **Kadowaki *et al.*, (2005)** mentioned searchers found polyphenols of green tea extract have potent inhibitors of the detoxification enzyme and **Sang *et al.*, (2005a)** mentioned catechins of green tea extract are known to be unstable at neutral and basic pH and undergo oxidation leading to production of reactive oxygen species. Besides, EGCG of green tea extract has been reported to have both antioxidant and pro-oxidative activities.

5.Conclusion:

The results obtained from the present study revealed that experimental pulmonary fibrosis induced by bleomycin shared most features with human pulmonary fibrosis. Therefore, these models seem to be adequate to verify green tea extract efficacy.

Administration of green tea extract pre and during induction of pulmonary fibrosis could largely reduce its pathological conditions than during and post induction.

Conflicts of interest:

There are no conflicts of interest.

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