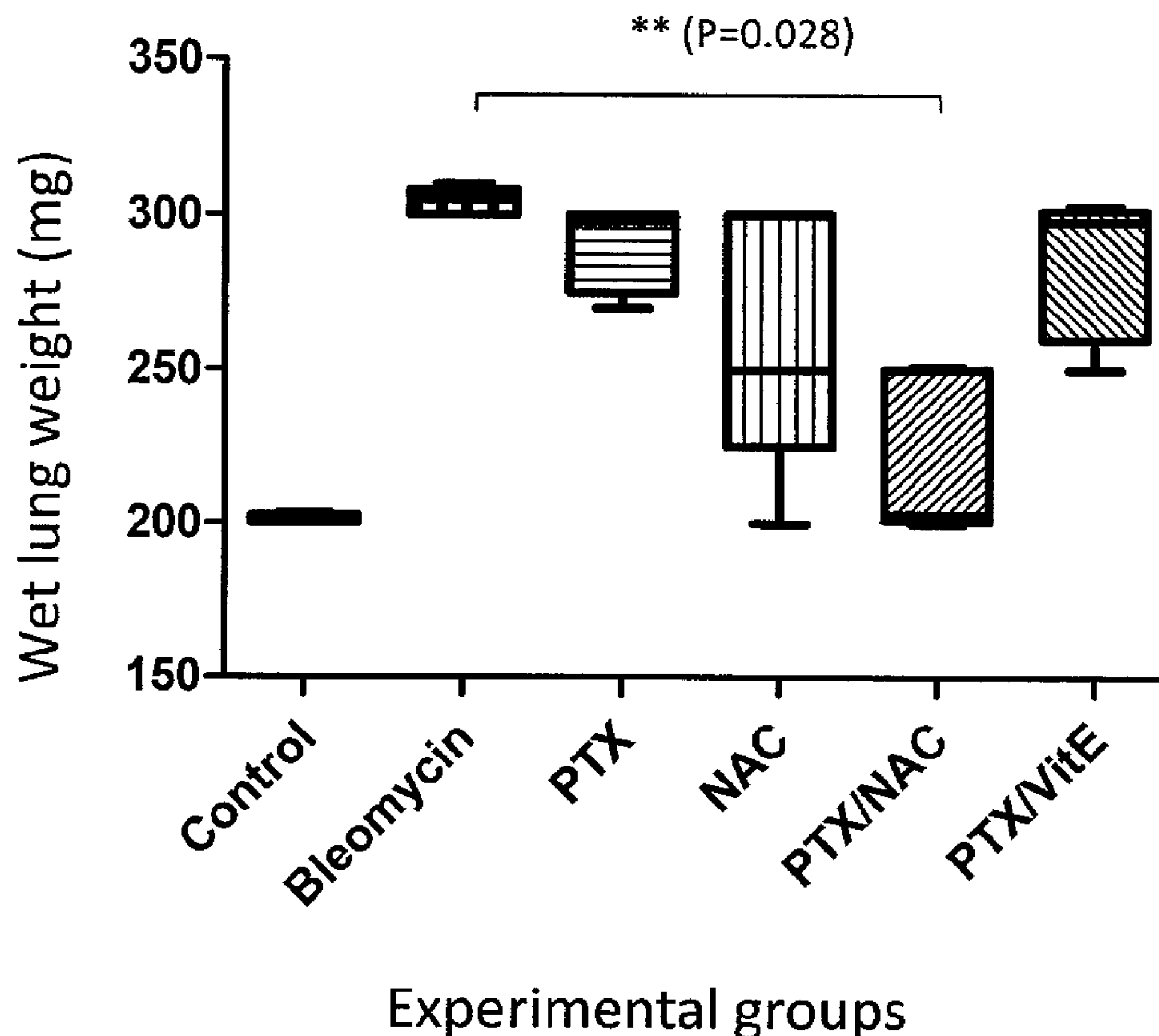




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(54) **Titre : COMPOSITIONS ET PROCEDES PERMETTANT DE TRAITER DES TROUBLES FIBROPROLIFERATIFS**
(54) **Title: COMPOSITIONS AND METHODS FOR TREATING FIBROPROLIFERATIVE DISORDERS**



(57) **Abrégé/Abstract:**

The present invention relates, in general, to fibroproliferative disorders, and, in particular, to methods and compositions for treating fibroproliferative disorders by administering to a mammal in need of treatment pharmacologically effective doses of a phosphodiesterase inhibitor, such as pentoxifylline, and an anti-oxidant which is a precursor of glutathione, such as N-acetyl-cysteine, or their derivatives or metabolites.

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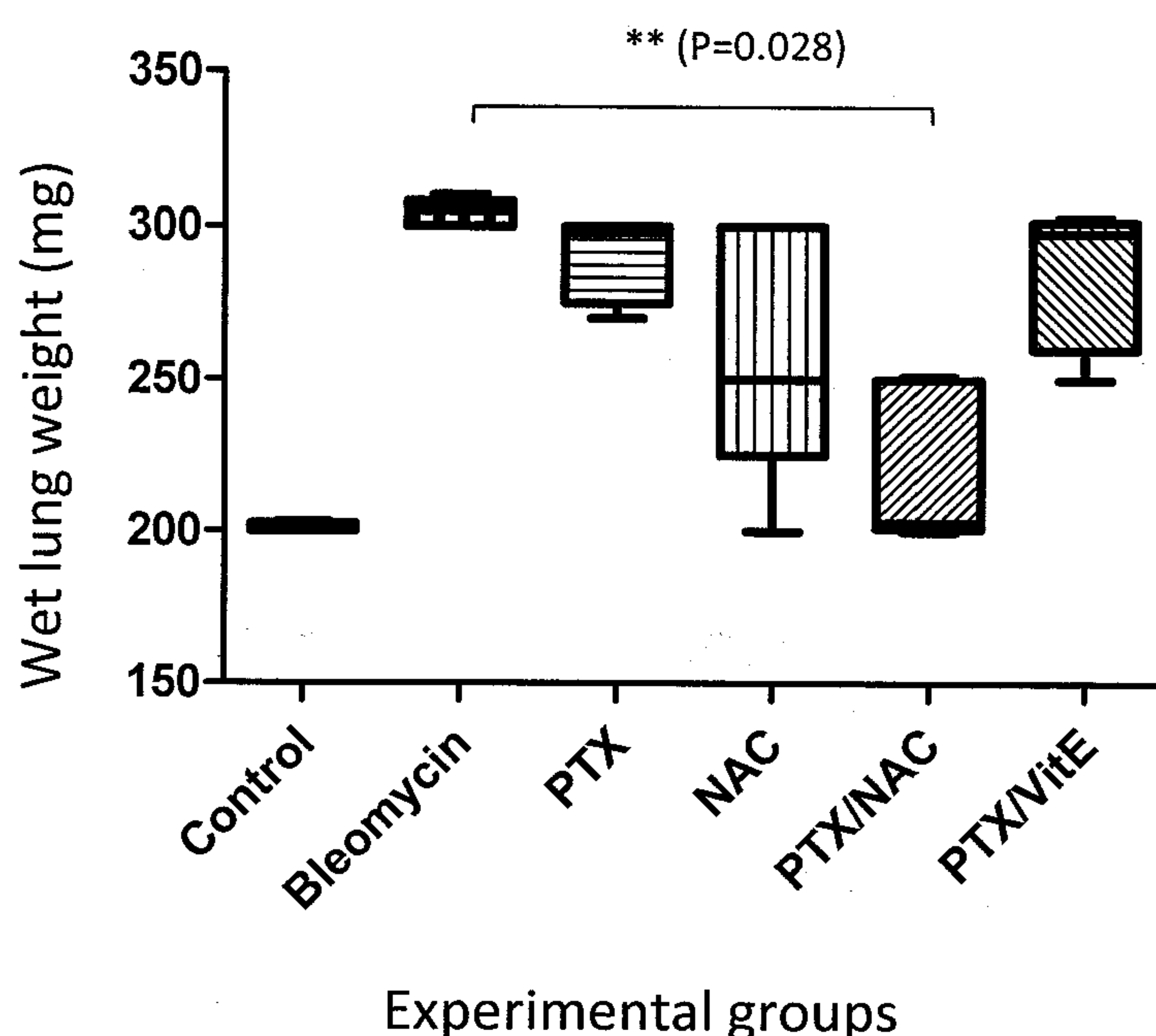
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(54) Title: COMPOSITIONS AND METHODS FOR TREATING FIBROPROLIFERATIVE DISORDERS



(57) Abstract: The present invention relates, in general, to fibroproliferative disorders, and, in particular, to methods and compositions for treating fibroproliferative disorders by administering to a mammal in need of treatment pharmacologically effective doses of a phosphodiesterase inhibitor, such as pentoxifylline, and an anti-oxidant which is a precursor of glutathione, such as N-acetyl-cysteine, or their derivatives or metabolites.

FIGURE 3

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COMPOSITIONS AND METHODS FOR TREATING
FIBROPROLIFERATIVE DISORDERS

5

10 Technical Field

[0002] This invention relates to fibroproliferative disorders. In particular, this invention relates to compositions and methods for treating fibroproliferative disorders.

15 Background

[0003] Tissue damage may result from physical injury, infection, exposure to toxins, auto-immune processes and other causes. The physiological process of normal wound repair after tissue injury involves inflammation, the recruitment, activation and proliferation of fibroblasts and the secretion of extracellular matrix. This response ordinarily culminates in healing and termination of the proliferative and secretory processes. However, in clinically diverse conditions, the fibroproliferative response becomes itself detrimental and produces an abnormal accumulation of fibrocellular scar tissue that further compromises the normal architecture and function of the affected tissue and, in time, becomes the main cause for morbidity and mortality in these conditions.

[0004] As shown schematically in Figure 1, injuries to normal tissues result in production of reactive oxidative species (ROS) and the release of cytokines, including TNF- α , PDGF, ET-1, bFGF, VEGF, TGF- β 1 and chemokines CCL2 and CCL7. Mononuclear cells (monocytes/macrophages and neutrophils), T-lymphocytes and

fibroblasts are recruited to the injury site. In response to released cytokines, these cells become activated and are a source for pro-fibrotic cytokines, namely TGF- β 1 and CTGF. "Activated" fibroblasts trans-differentiate into myofibroblasts which are collagen type I producing cells. The final result is aberrant tissue remodeling, fibrosis and permanent scarring.

[0005] Pathological fibrosis can occur in almost any organ or tissue in the body. Examples include, but are not limited to:

- 1) All forms of pulmonary fibrosis from coal miners' Black Lung Disease to the treatment-induced varieties occurring in cancer patients and premature babies. Typically fibrocellular scar tissue severely reduces lung diffusion capacity, vital capacity and progresses relentlessly to respiratory failure and death.
- 2) All forms of liver fibrosis and cirrhosis.
- 3) All forms of vascular fibrosis such as atherosclerosis and diabetic complications.
- 4) All forms of renal fibrosis.
- 5) All forms of interventional therapy triggered fibrosis such as restenosis of blood vessels after balloon angioplasties and atherectomies.

[0006] These fibroses are the cause of much suffering, disability and death in millions of patients across the world.

[0007] In recent years, due to growing understanding of biochemical and molecular events underlying the progression of fibrosis in whatever organ is affected, reasonable scientific strategies have been generated and, as a result, several experimental drugs for treatment or prophylaxis of fibrosis are in clinical trials and there is considerable optimism that additional new therapies will emerge in the years ahead.

[0008] Typically, treatment of fibroproliferative disorders comprises removal of the underlying cause (*e.g.*, toxin or infectious agent), suppression of inflammation (using, *e.g.*, corticosteroids and immunosuppressive agents such cyclophosphamide and azathioprine),

inhibition of fibroblast-like cell proliferation (using colchicines, penicillamine), down-regulation of cytokine machinery (using anti-TGF-beta antibodies, endothelin receptor inhibitors, interferons, pirfenidone and others), promotion of matrix degradation (using
5 inhibitors of matrix metalloproteinases), or promotion of fibroblast apoptosis. Despite recent progress, many of these strategies are still in the experimental stage, and existing therapies are largely aimed at suppressing inflammation rather than addressing the underlying biochemical processes. Thus, there remains a need for more effective
10 methods for treating fibroproliferative disorders.

[0009] Studies have demonstrated that pentoxifylline (PTX) is capable of positively effecting fibroproliferative disorders in a multi-potent manner. First, as a pan-phosphodiesterase inhibitor, pentoxifylline improves microcirculation and tissue oxygenation of the
15 fibrotic tissue [1-3]; second, pentoxifylline alters the biochemical and physical properties of platelets thus decreasing platelet aggregation in fibrotic tissues [4]; third, pentoxifylline exerts significant anti-cytokine and anti-inflammatory activity, as it is principally capable of inhibiting the pro-inflammatory actions of interleukin-1 (IL-1) and tumor necrosis
20 factor- α (TNF- α) on neutrophil function and cytokine production by monocytic cells [5]. Finally, pentoxifylline has shown direct inhibition of proliferation and collagen synthesis of human fibroblasts derived from normal and keloid skin and from hypertrophic scars [6]. Other phosphodiesterase inhibitors have some or all of the effects of
25 pentoxifylline [19, 20].

[0010] Use of pentoxifylline as the sole agent in the treatment of liver and other fibrosis is claimed in U.S. patent No. 5,985,592 to Peterson entitled Uses for Pentoxifylline or Functional
Derivatives/Metabolites Thereof. It is well known that fibroproliferative
30 disorders are characterized by an accumulation of immunomodulatory cells (macrophages and neutrophils), parenchymal injury, and fibrosis [7]. Those cells in the affected tissue release exaggerated amounts of

highly reactive oxygen radicals (oxidants), which mediate the parenchymal cell damage that typifies fibroproliferative disorders [21-24]. This oxidant burden is even more consequential due to a deficiency of glutathione, the major component of the antioxidant defence systems that normally protect against oxidant induced injury [8, 9].

[0011] In addition, low glutathione levels seem to play a major role in the exaggerated fibroblast proliferation seen in fibrosis [10]. Therefore, a rational therapeutic strategy for fibroproliferative disorders is to augment glutathione levels that would serve as protective screen to counterbalance toxic oxygen radicals.

[0012] For many years, N-acetyl-L-cysteine (NAC), a glutathione precursor, has been widely used as a mucolytic drug in pulmonary medicine [25]. The antioxidant potential of N-acetyl-L-cysteine has been established *in vitro* and *in vivo*. *In vitro*, the antioxidant capacity of N-acetyl-L-cysteine is directly related to the inactivation of electrophilic groups of free radicals [11, 12]. *In vivo*, N-acetyl-L-cysteine exerts its function as an antioxidant via its main metabolite, cysteine, the major precursor in the biosynthesis of glutathione [13]. In this respect, in paracetamol poisoning, oral N-acetyl-L-cysteine is able to replenish liver glutathione pools and to prevent drug-induced hepatotoxicity [14, 15]. In patients with lung tumours, oral treatment with N-acetyl-L-cysteine leads to an increase of glutathione levels in venous plasma and bronchoalveolar lavage fluid [16].

[0013] Oral N-acetyl-L-cysteine therapy in pulmonary fibrosis patients not only increased lung glutathione levels, but it did so with no short-term adverse effects. The therapy was safe, as judged by all routine clinical and bronchoscopic parameters evaluated [10].

Summary of the Invention

[0014] A method of treating fibroproliferative disorders and delaying disease progression associated therewith in a mammal is provided. The method comprises administering a composition

comprising a phosphodiesterase inhibitor in combination with an anti-oxidant which is a precursor of glutathione. In one embodiment, the phosphodiesterase inhibitor is pentoxifylline and the anti-oxidant is N-acetyl-L-cysteine. In another embodiment, the composition
5 comprises derivatives or metabolites of either or both of the phosphodiesterase inhibitor and the anti-oxidant.

[0015] In one embodiment, the method comprises administering a daily dose of pentoxifylline in the range of 10 mg/kg and a daily dose of N-acetyl-L-cysteine in the range of 20 mg/kg. In other embodiments,
10 the daily dose of pentoxifylline may be in the range of 200 mg to 1200 mg, and the daily dose of N-acetyl-L-cysteine may be in the range of 200 mg to 1800 mg. In some embodiments, the ratio of pentoxifylline to N-acetyl-L-cysteine may be in the range of 2:1 to 1:2. In one embodiment, the ratio of pentoxifylline to N-acetyl-L-cysteine is 1:2. A
15 pharmacologically effective dose of the phosphodiesterase inhibitor and a pharmacologically effective dose of the anti-oxidant are effective in combination to treat a fibroproliferative disorder. In some embodiments, the pharmacologically effective dose of the phosphodiesterase inhibitor and the pharmacologically effective dose of
20 the anti-oxidant are reduced respectively to a level below a pharmacologically effective dose of the phosphodiesterase inhibitor when the phosphodiesterase inhibitor is administered individually, and below a pharmacologically effective dose of the anti-oxidant when the anti-oxidant is administered individually.

[0016] A composition comprising a pharmacologically effective dose of a phosphodiesterase inhibitor and a pharmacologically effective dose of an anti-oxidant which is a precursor of glutathione is also provided. In some embodiments of the invention, the phosphodiesterase inhibitor is pentoxifylline and the anti-oxidant is N-acetyl-L-cysteine.
25 In other embodiments of the invention, the composition comprises derivatives or metabolites of either or both of the phosphodiesterase inhibitor and the anti-oxidant.
30

[0017] The composition may include the phosphodiesterase inhibitor and the anti-oxidant in a dosage unit form. In some embodiments, the dosage unit form may include in the range of 200 mg to 1200 mg pentoxifylline, and in the range of 200 mg to 1800 mg of N-acetyl-L-cysteine. In some embodiments, the ratio of pentoxifylline to N-acetyl-L-cysteine in the dosage form may be in the range of 2:1 to 1:2. In one embodiment, the ratio of pentoxifylline to N-acetyl-L-cysteine in the dosage form is 1:2. In some embodiments, the dosage form is suitable to administer a daily dosage of pentoxifylline in the range of 10 mg/kg, and to administer a daily dosage of N-acetyl-L-cysteine in the range of 20 mg/kg.

Brief Description of Drawings

[0018] In drawings which show non-limiting embodiments of the invention:

Figure 1 is a schematic diagram illustrating the typical physiological progression of a fibroproliferative disorder.

Figure 2 is a schematic diagram illustrating the scientific rationale for using pentoxifylline and N-acetyl-L-cysteine in combination to treat fibroproliferative disorders.

Figure 3 illustrates the effect of pentoxifylline and N-acetyl-cysteine on wet lung weight. Lung weight (mg) in experimental groups, as indicated. Data are representative of 4 independent experiments, presented as mean $\pm$ SD (n=5).

Figure 4 illustrates representative photomicrographs of lung histopathology. Normal lung histology is shown in panel A (Group I - saline). Panel B shows results for Group II (bleomycin only) while Panel C shows results for Group V (bleomycin plus a combination of PTX and NAC). Collagen is stained with Masson trichrome (light blue color). Original x10 and x40 magnifications are shown.

Figure 5 illustrates the effect of pentoxifylline and N-acetyl-cysteine on histological lesions scores of the extent and

severity of fibrosis in the experimental groups, as indicated. Data are representative of 5 independent experiments, presented as mean $\pm$ SD (n=4).

Figure 6 illustrates the effect of pentoxifylline and
5 N-acetyl-cysteine on a biochemical index of lung collagen accumulation -- hydroxyproline -- in the experimental groups, as indicated. Data are representative of 4 independent experiments, presented as mean $\pm$ SD (n=5).

Figure 7 shows results of TNF- α analysis (assayed by ELISA) in
10 bronchoalveolar lavage fluid (BALF) in different experimental groups, as indicated. Difference of TNF- α at day 5 post bleomycin instillation is shown. Values are means $\pm$ SD; 5 mice per group.

Figure 8 illustrates total glutathione levels in bronchoalveolar lavage fluid (BALF) in different experimental groups, as indicated.
15 Data are representative of 4 independent experiments, and presented as mean $\pm$ SD (n=5).

Description

[0019] Throughout the following description, specific details are
20 set forth in order to provide a more thorough understanding of the invention. However, the invention may be practiced without these particulars. In other instances, well known elements have not been shown or described in detail to avoid unnecessarily obscuring the invention. Accordingly, the specification and drawings are to be
25 regarded in an illustrative, rather than a restrictive, sense.

[0020] The present invention relates to methods and compositions for treating fibroproliferative disorders in mammals. In one embodiment, the method comprises administering to a mammal in need of such treatment an effective amount of a composition comprising
30 pentoxifylline and N-acetyl-L-cysteine.

[0021] The terms "treat" and "treatment" are used broadly to denote therapeutic and prophylactic interventions that favorably alter a

pathological state. Treatments include procedures that moderate or reverse the progression of, reduce the severity of, prevent, or cure a disease. As used herein, the term "fibroproliferative" includes all forms of pulmonary fibrosis (idiopathic, occupational and
5 environmental, auto-immune, scleroderma, sarcoidosis, drug- and radiation-induced, genetic/familial fibrosis); all forms of liver fibrosis and cirrhosis; all forms of kidney fibrosis, all forms of uterine fibrosis; all forms of vascular fibrosis such as atherosclerosis and diabetic complications; all forms of interventional therapy triggered fibrosis such
10 as restenosis of blood vessels after balloon angioplasties and atherectomies.

[0022] Preferred active agents for use in the present invention include pentoxifylline (or a pharmaceutically acceptable derivative or metabolite thereof, for example those described by Fantin et al. (2006)
15 [28]). However, other phosphodiesterase inhibitors may also be used. Examples of such compounds are sildenafil, vardenafil, and tadalafil [29]. While the use of N-acetyl-L-cysteine is also preferred, other precursor compounds that replenish glutathione concentration in bronchio-alveolar lavage can be used. Pharmaceutically acceptable
20 derivatives or metabolites of N-acetyl-L-cysteine may also be used.

[0023] As shown schematically in Figure 2, the combination of pentoxifylline and N-acetyl-L-cysteine may be used in some embodiments of the present invention. Pentoxifylline, a known pan-phosphodiesterase inhibitor, works by increasing the intracellular
25 level of cyclic adenosine monophosphate (cAMP), an important second messenger molecule in variety of cells. In several *in vitro* and *in vivo* systems, pentoxifylline has been shown to inhibit actions of some pro-fibrotic key cytokines including TNF- α [30, 31] and PDGF [32]. Moreover, pentoxifylline exerts strong inhibitory effects on both
30 fibroblast proliferation and trans-differentiation of "activated" fibroblasts into myofibroblasts [33-36]. Myofibroblasts are cells that produce excessive extracellular matrix protein, collagen type I. In

addition, pentoxifylline is capable of inhibiting collagen type I synthesis and promoting collagen type I degradation [35, 37-39]. These anti-fibrotic features of pentoxifylline as well as its known anti-ischemic property via vasodilation, coupled with low systemic toxicity, make
5 pentoxifylline an attractive drug candidate for treatment of fibroproliferative disorders. However, other phosphodiesterase inhibitors such as sildenafil, vardenafil, and tadalafil may also be used in accordance with the present invention.

[0024] Still with reference to Figure 2, N-acetyl-L-cysteine, a
10 glutathione precursor and strong anti-oxidant, complements the anti-fibrotic effects of pentoxifylline by countering reactive oxygen radicals generated throughout the disease progression, thus helping to protect tissues from being further damaged. While reference is made to N-acetyl-L-cysteine, other precursors of glutathione (for example, NAC
15 amide, cysteine esters, gammaglutamylcysteine and its ethyl ester, glutathione derivatives such as glutathione monoester, glutathione diester, lipoic acid and derivatives thereof) may also be used in accordance with the present invention. The final result of pentoxifylline and N-acetyl-L-cysteine combination treatment is retardation of
20 fibroproliferative disorders.

[0025] The amount of active agents (*e.g.*, pentoxifylline and N-acetyl-L-cysteine) administered can vary with the patient, the route of administration and the result sought. Optimum dosing regimens for particular patients can be readily determined by one skilled in the art.
25 For example, the daily dose of pentoxifylline can be from about 200 mg to about 1200 mg combined with a daily dose of N-acetyl-L-cysteine from 200 mg to about 1800 mg. In one embodiment, the daily dose of pentoxifylline may be in the range of 10 milligrams per kilogram of body weight, and the daily dose of N-acetyl-L-cysteine may be in the
30 range of 20 milligrams per kilogram of body weight. The ratio of pentoxifylline to N-acetyl-L-cysteine can range, for example, from about 2:1 to about 1:2.

[0026] The individual components of the composition can be administered separately at different times during the course of therapy or concurrently in divided or single combination forms.

[0027] In accordance with the present invention, the active agents
5 (which may be, for example, pentoxifylline and N-acetyl-L-cysteine) can be administered in any convenient manner, such as orally, by inhalation intranasally, sublingually, rectally, vaginally, parenterally (including subcutaneously, intrathecally, intramuscularly or intravenously), cutaneously, or transdermally.

10 **[0028]** The active agents of the invention can be administered in the form of a pharmaceutical composition or compositions that contain one or both in an admixture with a pharmaceutical carrier. The composition may be formulated for topical or systemic administration. The pharmaceutical composition can be in dosage unit form such as
15 tablet, capsule, sprinkle capsule, pill, granule, powder, syrup, suspension, emulsion, solution, gel including hydrogel, paste, ointment, cream, lotion, plaster, drench, suppository, enema, implant, spray or aerosol, suppository, injection or the like. Sustained released formulations can also be used. The composition can also be present in a
20 transdermal delivery system, which may be, by way of example, a skin patch. A large variety of delivery vehicles for administering the composition are contemplated as within the scope of the present invention when containing therapeutic amounts of PDE inhibitor (for example, pentoxifylline) and antioxidant (for example, NAC). Suitable
25 delivery vehicles include, but are not limited to, microcapsules or microspheres; liposomes and other lipid-based release systems; absorbable and/or biodegradable mechanical barriers, polymeric or gel-like materials.

[0029] In some embodiments, the dosage form may provide a
30 dosage of between 200 to 1200 mg of pentoxifylline, and a dosage of between 200 mg and 1800 mg of N-acetyl-L-cysteine. The ratio of pentoxifylline to N-acetyl-L-cysteine in the dosage form may be in the

range of 2:1 to 1:2. The dosage form may also be formulated to provide a daily dosage of pentoxifylline in the range of 10 milligrams per kilogram of body weight, and a daily dosage of N-acetyl-L-cysteine in the range of 20 milligrams per kilogram of body weight.

5 [0030] The pharmaceutical compositions may be formulated according to conventional pharmaceutical practice. Sustained release formulations can also be used. Each compound of the combination may be formulated in a variety of ways that are known in the art. For example, the first agent (PDE inhibitor) and the second agent
10 (anti-oxidant) may be formulated together or separately. Desirably, the two components are formulated together for simultaneous administration. Such co-formulated compositions can include the two agents formulated together in the same pills, capsule, liquid, etc. The individually or separately formulated agents can be packaged together as
15 a co-packaged product. Non-limiting examples include two pills, a pill and a powder, a suppository and a liquid in a vial, two topical creams, etc.

[0031] In accordance with the invention, the composition of pentoxifylline and N-acetyl-L-cysteine (including sustained release
20 preparations) is an effective treatment for fibroproliferative disorders and provides an effective means of delaying disease progression associated with fibrosis. The composition can be more effective than, for example, pentoxifylline or N-acetyl-L-cysteine treatment alone and with fewer side effects. Lower doses of both types of medication can be
25 used in the compound treatment, thereby further reducing the overall side effect burden. It is a particular advantage that, because of synergistic and superadditive effect on administration, the amounts of pentoxifylline and N-acetyl-L-cysteine which are to be administered can be reduced to those amounts which, on administration alone, show only
30 minimal pharmacological side effects (i.e. the side effects which are elicited by high doses of these medicaments can be avoided or diminished). This is of great importance because it is known that N-

acetyl-L-cysteine can, in the customary doses, elicit undesired side effects such as nausea, vomiting, headache, dry mouth, dizziness, or abdominal pain [17]. Moreover, pentoxifylline may show undesired side effects in the forms of malaise, flushing, dizziness/light-headedness, headache, nausea, vomiting, abdominal discomfort, bloating, diarrhea, dyspepsia [1, 18]. By means of the methods and compositions according to the invention it is now possible to reduce the dose of N-acetyl-L-cysteine necessary for mammals, including humans, as well as the amount of pentoxifylline, so that there is an even greater improvement in the general toxicological tolerability.

Example 1.0 *In vivo* investigations in a mouse model

Methods

[0032] The composition including pentoxifylline (PTX) and N-acetyl-cysteine (NAC) was assessed *in vivo* in a mouse model using an experimental design in which a pulmonary fibrosis was generated using bleomycin injury of lung. Bleomycin (BLM) is an effective antineoplastic drug that binds to and damages DNA of tumour cells. However, administration of BLM may result in lung inflammation that can progress to fibrosis. This side effect is due mostly to augmented concentration of reactive oxygen species, decrease in nicotinamide adenine dinucleotide (NAD) and adenosine triphosphate (ATP), and overproduction of mature collagen fibrils. Because BLM-induced lung fibrosis is easily reproduced in different species of mammals, experimental models using the drug have been adopted with the goal of investigating the efficacy of the current invention [45].

[0033] From the literature, both N-acetyl cysteine (NAC) and pentoxifylline (PTX) individually have been shown to have efficacy in the bleomycin-induced lung injury animal model [40-44]. In this example, the effects of PTX and NAC in combination were studied in comparison to PTX and NAC individually. A comparison between the efficacy of the PTX/NAC combination vs. the combination of PTX and

Vitamin E (another antioxidant) was also included in some experiments. The test substances were administered as shown in Table 1.

Table 1: Experimental Design of Bleomycin-Induced Pulmonary Fibrosis Mouse Model Study

Group No.	Treatment	No. of animals	Test article (volume/dose); route of administration
I	Vehicle (negative) control	5-20	Saline (50 μ l); IT Saline (100 μ l); IP
II	BLM (positive) control	5-20	BLM (50 μ l/0.06 U); IT Saline (100 μ l); IP
III	BLM + PTX	5-20	BLM (50 μ l/0.06 U); IT PTX (100 μ l/10 mg/kg); IP
IV	BLM + NAC	5-20	BLM (50 μ l/0.06 U); IT NAC (100 μ l/20 mg/kg); IP
V	BLM + PTX/NAC combination	5-20	BLM (50 μ l/0.06 U); iIT PTX (50 μ l/10 mg/kg); IP and NAC (50 μ l/20 mg/kg); IP
VI	BLM + PTX/Vitamin E combination	5	BLM (50 μ l/0.06 U); IT PTX (50 μ l/10 mg/kg); IP and Vitamin E (50 μ l/1.5 mg); IP

Abbreviations: IP, Intraperitoneal; IT, Intratracheal; NAC, N-acetylcysteine; No., Number; PTX, Pentoxifylline; BLM, Bleomycin

[0034] Mice (under anesthesia) received a single intratracheal dose of either saline (control) or bleomycin (BLM). The mice were administered intraperitoneal (IP) doses of saline, PTX, NAC, or Vitamin E and combinations thereof, daily for 14 days. On the appropriate day, the mice were given surgically deep anesthesia, total blood was obtained by cardiac puncture, bronchoalveolar lavage fluid (BALF) was collected from the lungs for further analysis of TNF- α and total glutathione (GSH) levels. In addition, the lung weights were recorded, photographs of the lungs were taken and tissue samples processed either for biochemical analysis of hydroxyproline (immediately frozen on dry ice) or further morphological analysis (immersed in fixative solution of 10% neutral-buffered formalin).

Results

[0035] As anticipated, BLM (Group II) produced a significant increase in lung weight. In mice exposed to BLM and subsequently treated with PTX or NAC alone (Groups III and IV, respectively), lower lung weights were observed but statistical significance was not reached. However, when PTX and NAC were used in combination following bleomycin instillation (Group V), a statistically significant decrease in lung weight was observed (see Figure 3 -- fourteen days treatment of the mice exposed to bleomycin resulted in lower lung weights but statistical significance was reached when PTX (pentoxifylline) and NAC (N-acetyl-cysteine) were used in combination).

[0036] Lungs from mice in control vehicle group (vehicle + vehicle) were histologically normal. Fourteen days after intratracheal bleomycin, lungs from mice in Group II (BLM + saline) showed marked peri-bronchiolar and interstitial infiltration with inflammatory cells, extensive cellular thickening of intra-alveolar septa, interstitial oedema, increases in interstitial cells with a fibroblastic appearance and in interstitial collagen deposition detected by the trichrome stain (i.e. fibrosis) (see Figure 4; Panel B). These pulmonary lesions were markedly reduced in animals treated with the combination of pentoxifylline and NAC (Group V - bleomycin plus a combination of PTX and NAC - panel C). In Groups IV (BLM + NAC) and V (BLM + NAC and PTX), there was a marked anti-fibrotic effect seen, and although multifocal parenchymal lesions were still present in lungs, the organized foci were less frequent and smaller than those seen in untreated animals, showed less oedema and collagen deposition, and less septal widening and clusters of inflammatory cells (see Figure 4; Panel C for Group V).

[0037] The semi-quantitative score of the severity and extent of inflammation and fibrosis showed that most of indices were reduced in

Group V (BLM + NAC and PTX), statistical significance was reached for scores related to severity and extension of fibrosis (see Figure 5). The treatment combination of PTX and NAC had significant inhibitory effects on the severity and extent of BLM-initiated lung pathology. The
5 PTX + NAC treatment was more effective than PTX or NAC alone and the combination of PTX + Vitamin E on the induced fibrosis in this animal model.

[0038] The extent of pulmonary fibrosis was also assessed biochemically. Lung hydroxyproline content was determined as an
10 index of parenchymal collagen accumulation. Figure 4 shows that intratracheal instillation of BLM induced a significant increase in total lung collagen compared with saline treatment. Administration of PTX and NAC combination therapy to bleomycin treated mice attenuated this increase by approximately 37% (see Figure 6). Co-administration of
15 PTX and NAC resulted in statistically significant reduction of the lung content of hydroxyproline.

[0039] To demonstrate that treatment with PTX and NAC alters cytokine release during the course of BLM-induced lung fibrosis, the inventors evaluated the release or secretion of proinflammatory cytokine
20 TNF- α in the bronchoalveolar lavage fluid (BALF) using a commercially available ELISA kit. Treatment with either PTX or NAC significantly decreased BLM-induced increases in TNF- α level. Co-administration of PTX and NAC resulted in super-additive and synergistic effect (see Figure 7). This beneficial effect of the
25 combination can be explained by the reported ability of PTX to directly modulate production of TNF- α by inflammatory cells; and NAC's antioxidant ability to detoxify BLM-generated radicals before they damage lung tissue. In addition to a direct scavenging ability of BLM-generated oxygen radicals, NAC has protective effects through
30 glutathione synthesis as a known precursor of glutathione (GSH). The inventors' data indicates that GSH is decreased in the BLM group, and that GSH levels can indeed be supplemented by daily administration of

NAC (see Figure 8). Therefore, the super-additive therapeutic effect observed with the combination therapy of PTX plus GSH can be also attributed to the daily supplementation of GSH, major lung anti-oxidant, in the alveolar space, thus helping to attenuate the BLM-induced lung injury.

5 [0040] Moreover, the inventors found no deaths or abnormal reactions with a daily co-administration of PTX and NAC during the experiments. The *in vivo* investigation of the combination therapy in a mouse model demonstrates that the therapy is likely a safe and effective method of treatment of pulmonary fibrosis in mammals.

10 [0041] As will be apparent to those skilled in the art in the light of the foregoing disclosure, many alterations and modifications are possible in the practice of this invention without departing from the spirit or scope thereof. It is therefore intended that the following
15 appended claims and claims hereafter introduced are interpreted to include all such alterations and modifications as are within their true scope.

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WHAT IS CLAIMED IS:

1. Use of a combination of pentoxifylline and N-acetyl-L-cysteine in the treatment of a fibroproliferative disorder in a mammal.
- 5 2. The use according to claim 1, wherein the pentoxifylline and the N-acetyl-L-cysteine are adapted to be administered separately.
- 10 3. The use according to claim 1, wherein the pentoxifylline and the N-acetyl-L-cysteine are adapted to be administered concurrently.
4. The use according to claim 1, wherein the daily dose of pentoxifylline is in the range of 200 mg to 1200 mg; and wherein the daily dose of N-acetyl-L-cysteine is in the range of 200 mg to 1800 mg.
- 15 5. The use according to claim 1, wherein the daily dose of pentoxifylline is 10 mg/kg, and wherein the daily dose of N-acetyl-L-cysteine is 20 mg/kg.
- 20 6. The use according to claim 4, wherein the ratio of the pentoxifylline to the N-acetyl-L-cysteine is in the range of 2:1 to 1:2.
7. The use according to claim 6, wherein the ratio of the pentoxifylline to the N-acetyl-L-cysteine is 1:2.
- 25 8. The use according to any one of claims 1 to 7, wherein both the pentoxifylline and the N-acetyl-L-cysteine are adapted for oral, parenteral, rectal, cutaneous, nasal, vaginal, or inhalant use.

9. The use according to any one of claims 1 to 8, wherein either or both of the pentoxifylline and the N-acetyl-L-cysteine are admixed with a pharmaceutical carrier before administration.

5

10. The use according to any one of claims 1 to 9, wherein the fibroproliferative disorder is one of pulmonary fibrosis, liver fibrosis, kidney fibrosis, uterine fibrosis, vascular fibrosis including peripheral arterial disease, or interventional therapy triggered fibrosis.

10

11. A composition comprising a pharmacologically effective dose of pentoxifylline and a pharmacologically effective dose of N-acetyl-L-cysteine which is a precursor of glutathione, for use in the treatment of a fibroproliferative disorder.

15

12. A composition comprising a combination of pentoxifylline and N-acetyl-L-cysteine in a dosage unit form.

20

13. A composition according to claim 12, wherein the dosage unit form is in the form of a tablet, capsule, granule, powder, syrup, suspension, emulsion, solution, gel, paste, ointment, cream, lotion, plaster, skin patch, drench, suppository, enema, injectable, implant, spray or aerosol.

25

14. A composition according to claim 13, wherein the dosage unit form comprises in the range of 200 mg to 1200 mg pentoxifylline, and in the range of 200 mg to 1800 mg of N-acetyl-L-cysteine.

15. A composition according to claim 12 or 14, wherein the ratio of pentoxifylline to N-acetyl-L-cysteine in the dosage form is in the range of 2:1 to 1:2.
- 5 16. A composition according to claim 15, wherein the ratio of pentoxifylline to N-acetyl-L-cysteine in the dosage form is 1:2.
17. A composition according to claim 12 wherein the dosage form is suitable to administer a daily dosage of pentoxifylline in the range of 10 mg/kg, and
10 suitable to administer a daily dosage of N-acetyl-L-cysteine in the range of 20 mg/kg.
18. A composition according to any one of claims 11 to 17, further comprising a pharmaceutically acceptable carrier.
- 15 19. A composition according to claim 12, wherein the dose of pentoxifylline and the dose of N-acetyl-L-cysteine are effective in combination to treat a fibroproliferative disorder.

Tissue injury: oxidative stress (radiation, pollutants, bad diet), auto-antibodies (scleroderma), viral injury, physical trauma, unidentified insult (IPF), etc.

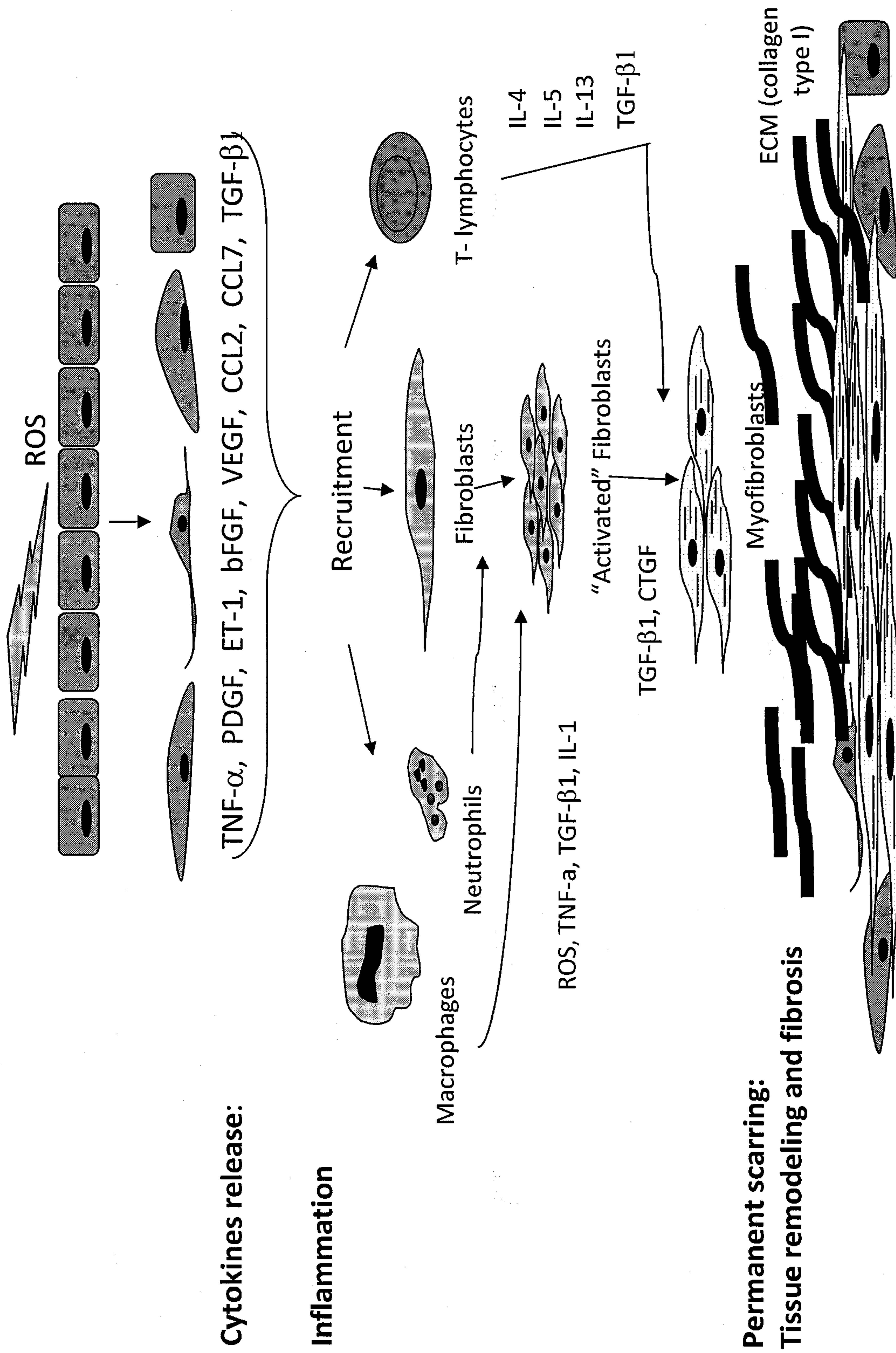
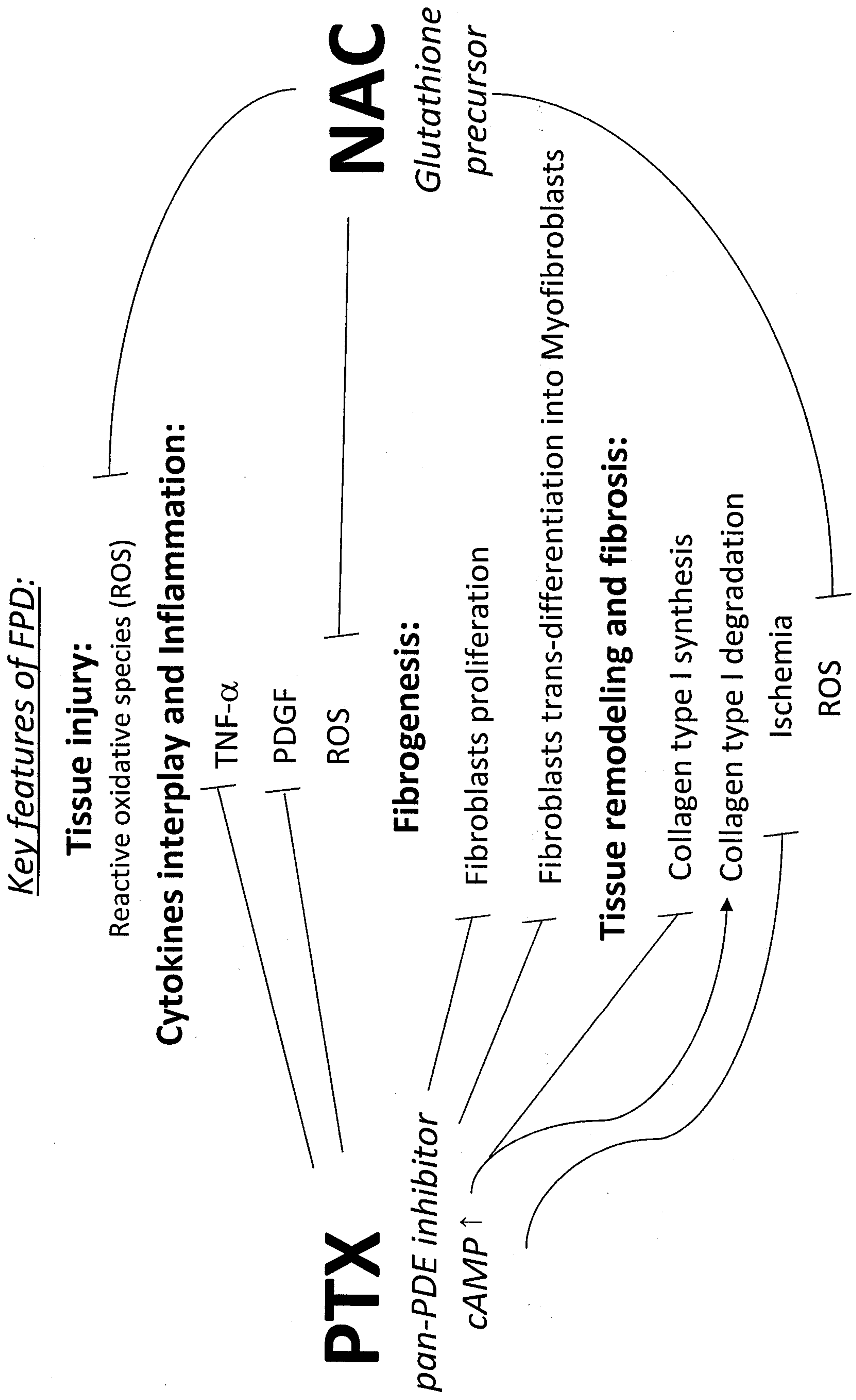


FIGURE 1



PTX + NAC combo = FPD retardation

FIGURE 2

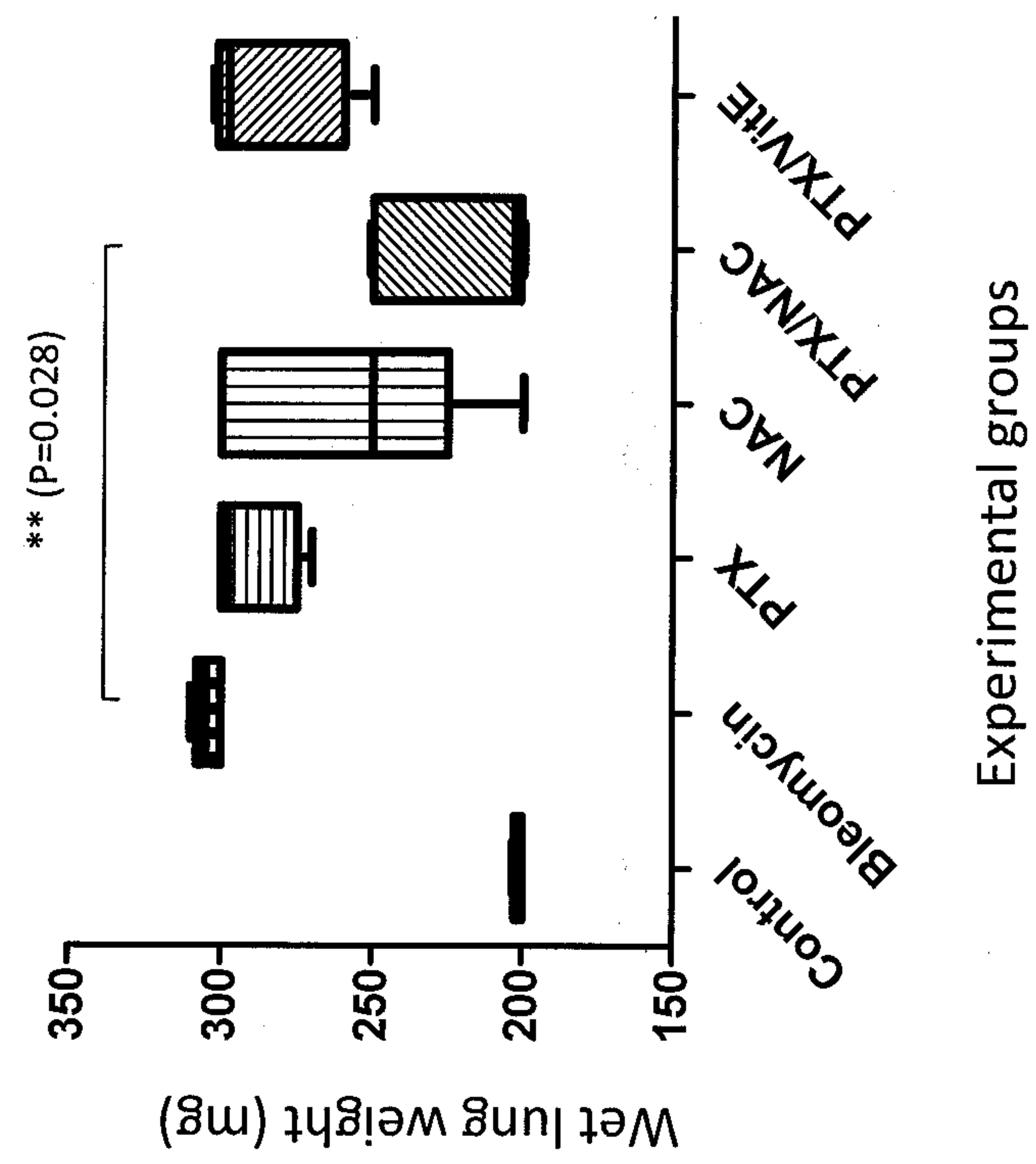


FIGURE 3

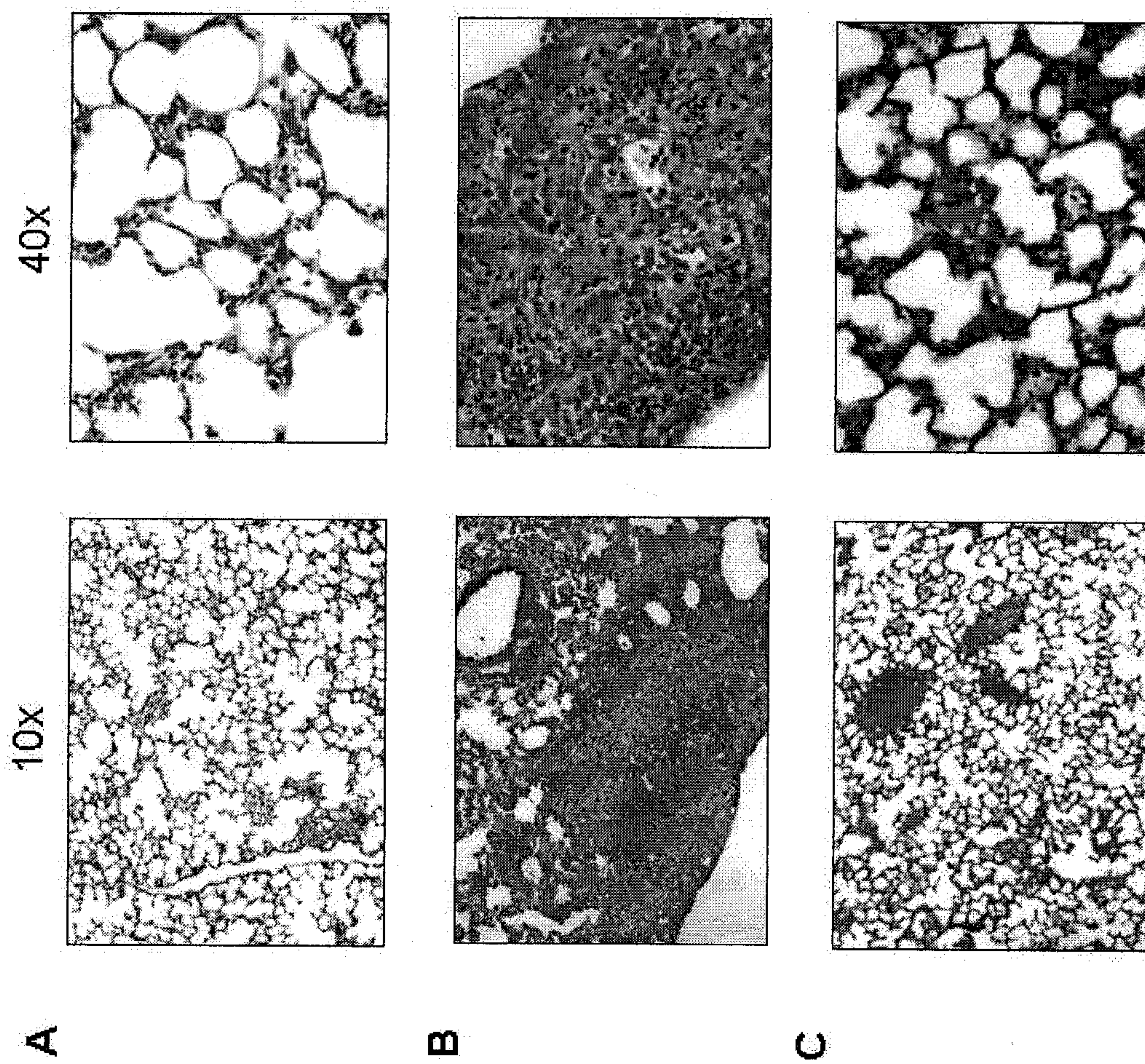


FIGURE 4

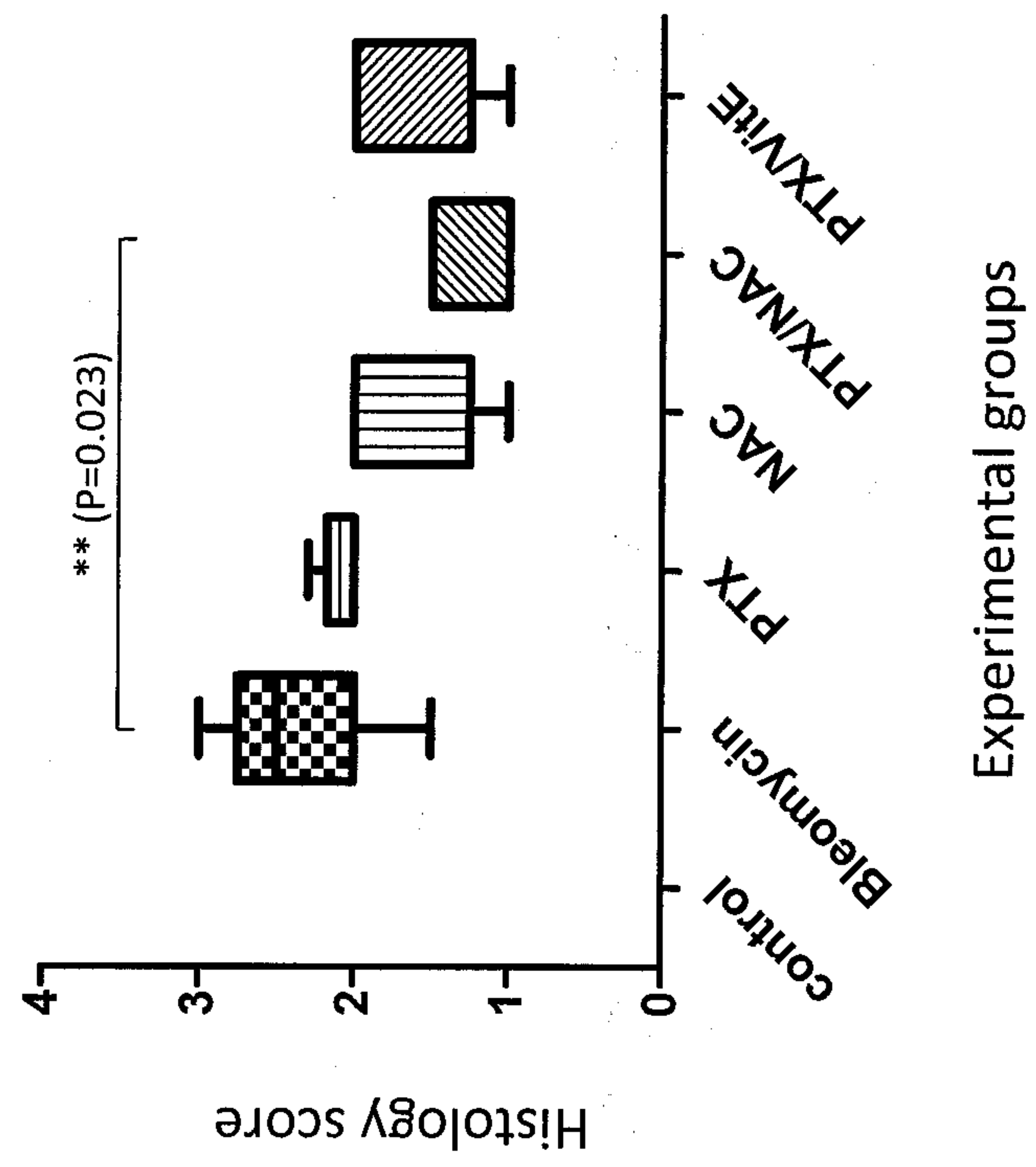


FIGURE 5

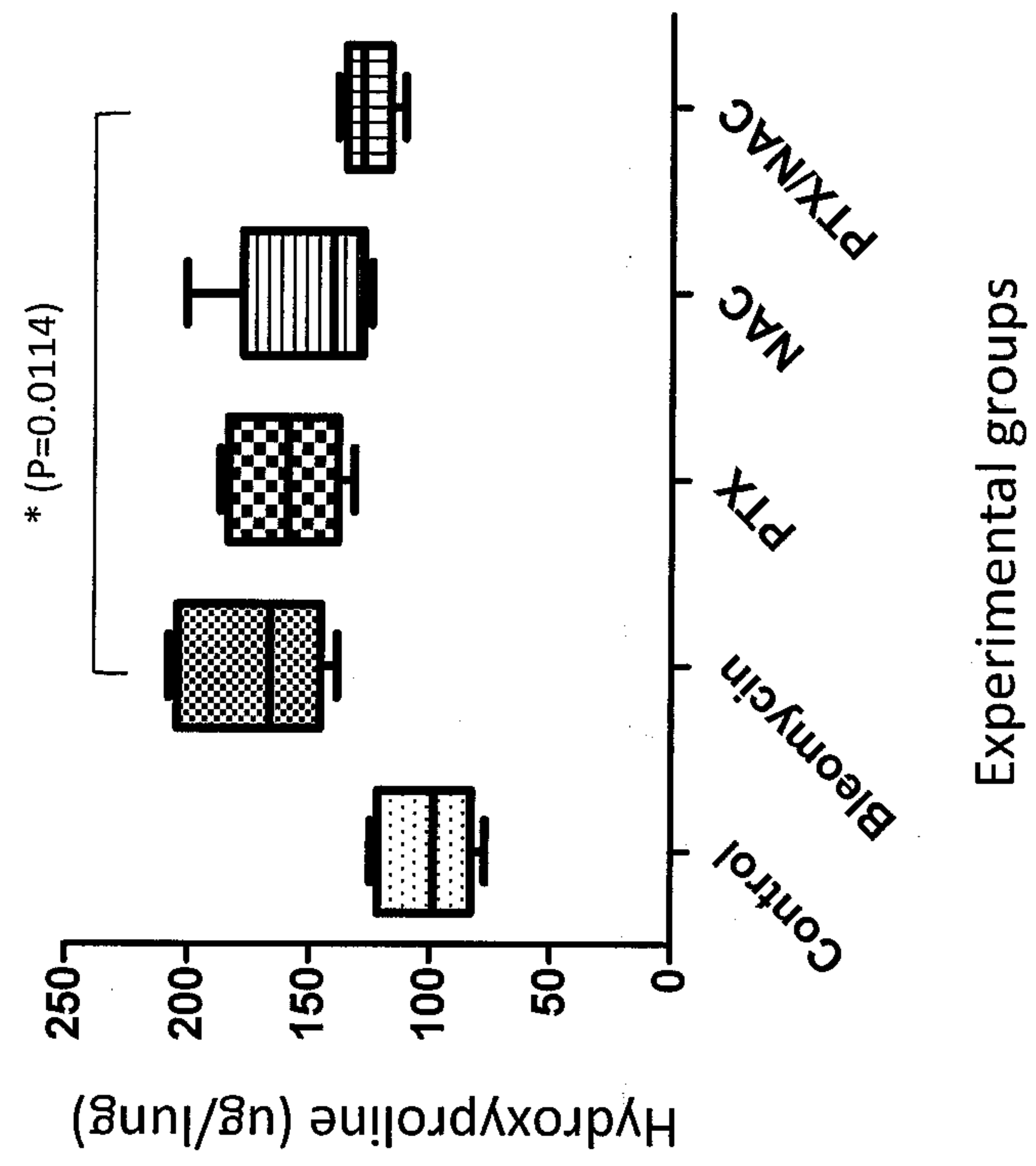


FIGURE 6

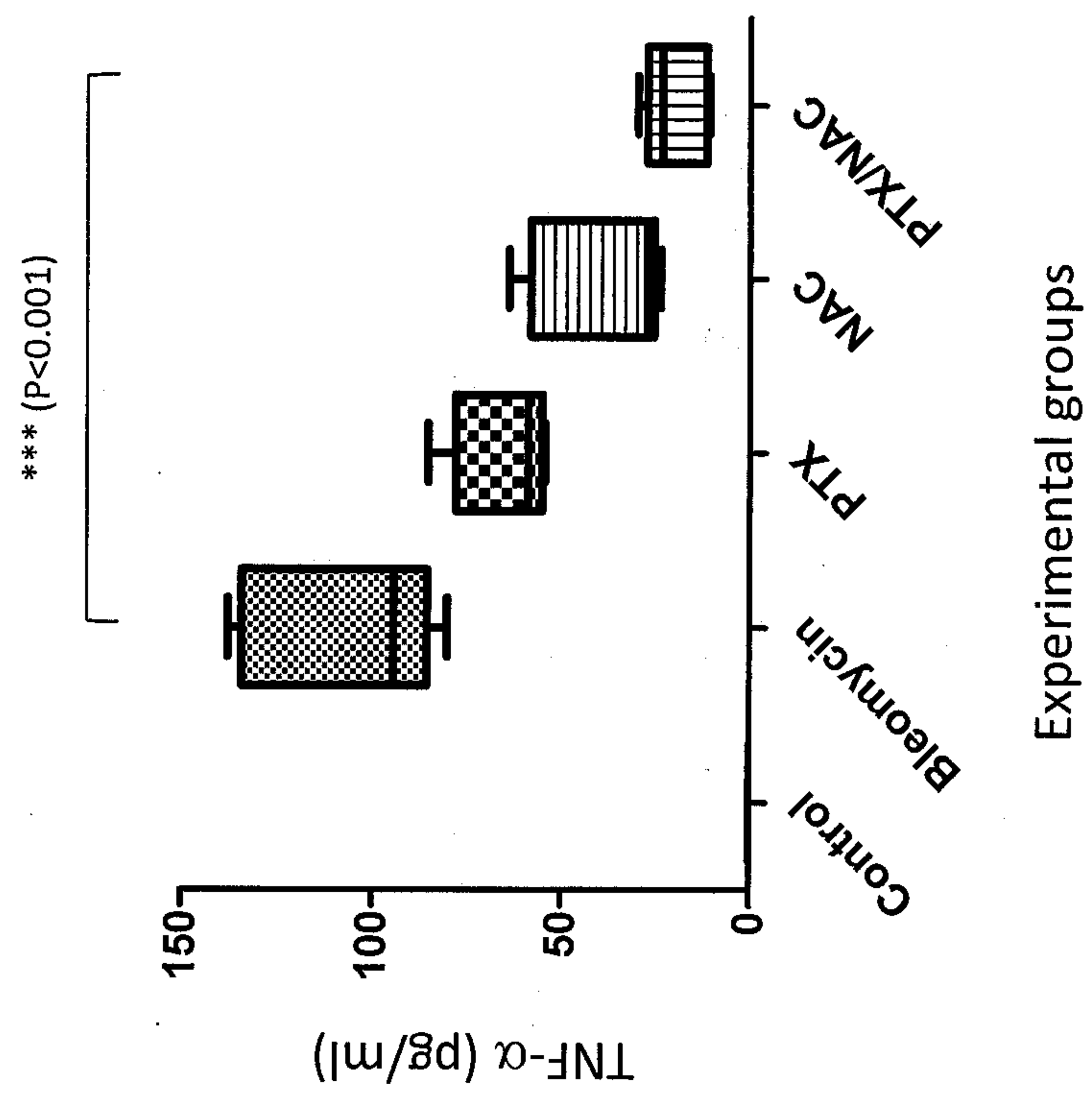


FIGURE 7

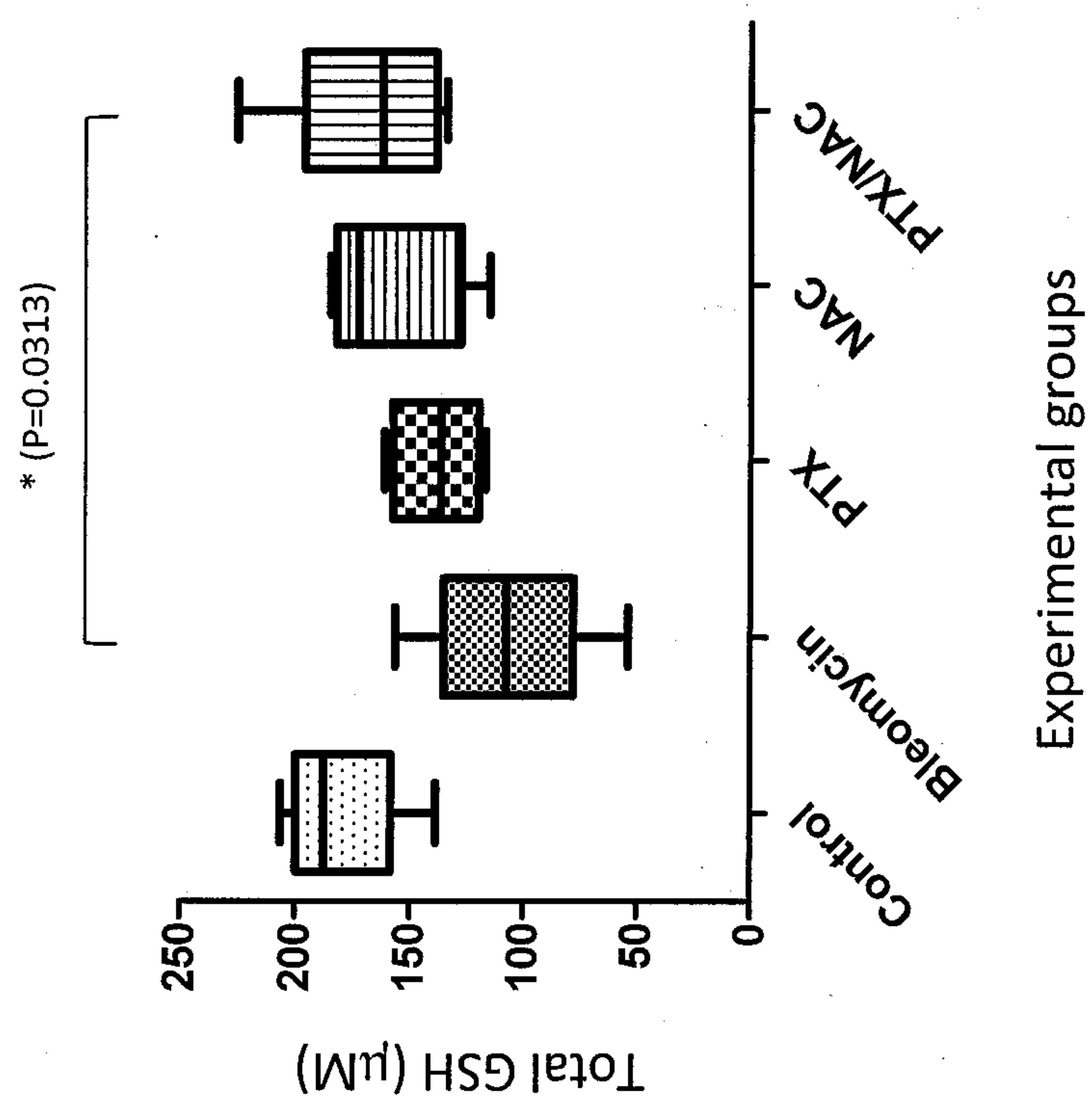


FIGURE 8

Wet lung weight (mg)

