THE ROLE ARACHIDONIC ACID METABOLITES IN THE RESPONSE OF GUINEA PIG LUNG AND DERMALVASCULATURE TO EXTRACTS OF COTTON DUST

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Abstract

The ability of cotton dust extract (CDE) to release Inflammatory mediators have been considered to play a role in the development of byssinosis. In this study, an experimental model of guinea pig lung perfusion has been employed to examine the effect of previous exposure to CDE on the pulmonary perfusion pressure (PPP) and to study the release and pulmonary activity of arachidonic acid (AA) metabolites. In addition, the possibility of CDE acting as a sensitizing agent has been examined using a cutaneous reaction test.

CDE produced a significant increase in both PPP and cutaneous vascular permeability. In addition prostaglandin (s) and leukotriene (s) were released by CDE and appeared to be responsible for the pharmacological responses recorded. Prior exposure of the animals to CDE increased the subsequent responses of lung and skin to CDE challenge and mediator release was enhanced.

Introduction

Byssinosis, a respiratory disease of workers on cotton, flax and hemp, is classically characterized by shortness of breath, cough and chest tightness. Although the pathogenesis of byssinosis is not yet clear, the release of bronchoconstrictor agents from the lung by cotton dust or its extract has been proposed to play an important role (Bouhuys and Van de Woestigne, 1970).

The ability of acute cotton dust exposure to release arachidonic acid metabolites from the alveolar macrophages and perfused lung of guinea pig has been previously reported (El-Mahdy et al., 1985). Arachidonic acid metabolites such as the cysteinyl leukotrienes leukotriene C_4 (LTC_4) and LTD_4, are considered to be important mediators of allergic and anaphylactic reactions (Henderson, 1991). Leukotriene C_4 is also known to constrict airway and vascular smooth muscle from the lungs of several species (Piper, 1984). The responses to leukotrienes (LTs) may be mediated indirectly, in part by the release of other vasoactive compounds such as prostaglandins (Pgs) and thromboxane A_2 (TXA_2) (Engineer et al., 1978). The decrease in the ventilatory capacity of human lungs following the inspiration of cotton dust has been found to correlate more closely with the concentration of endotoxin in the dust (Elissalde and Beier, 1990). The endotoxin isolated from Enterobacter agglomerans, a common bacterial contaminant of cotton fibre, stimulates rat macrophages to release PGs and TXA_2 (Elissalde and Beier, 1990). It has been reported that inhalation of cotton dust extract (CDE) by rabbits causes a significant release of prostaglandin F_{2a} (PGF_{2a}) and TXA_2 into the alveolar space (Mundie and Ainsworth, 1985).

The in vitro techniques used to study reaction of airway smooth muscle rely almost entirely on tonic measurements of isolated guinea pig trachea (Burka and Paterson, 1981) and lung parenchmal strips (Chand, 1979). However, the only technique in which structural integrity of the airways and hence the elastic forces is maintained, is the vascular perfused lung.

In view of such considerations, it was decided that the use of the technically simpler preparation of the guinea pig isolated lung perfused through the pulmonary airways with CDE as a reasonably suitable model for investigating the effect of CDE on the release of arachidonic acid metabolite from lung tissues. In addition, the effect of CDE in the presence of different inhibitors of arachidonic acid metabolite synthesis on pulmonary perfusion pressure (PPP) was examined.

Since atopy has been shown to be of considerable value in the degree of acute bronchoconstriction after exposure to cotton dust (Sepulveda et al., 1984), the second part of this study was carried out to investigate the effect of CDE on the vascular permeability of skin as a marker of such activity. The results were compared with those obtained from animals exposed to a known sensitizing agent such as egg albumin (EA).

Materials and Methods

Preparation of Aqueous Cotton Dust Extract (CDE)

A 5 gm sample of raw cotton dust (Mehalla spinning & weaving company, Mehalla El-Kobra, Egypt) was ground to reduce the particle size. The dust powders were then macerated with 25 ml of saline while shaking mechanically in a shaker at a speed of 30 strokes/min. at room temperature for 2.5 h. After centrifugation at 3000 rpm for 25 min, the supernatant was removed and filtered.

Animals

Normal healthy guinea pigs (Egyptian bred) of mixed sex weighing 250-350 gm were used in this study. Animals were divided into 2 main cohorts as follows:

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Cohort 1

These animals were used to evaluate the effect of CDE on the perfused guinea pig lung and the production of PG and LT-like substances. Animals were further divided into three equal groups (five animals each). Treatment and sensitization of animals with CDE and EA were carried out according to the previously described method (Hitchcock and Kokolis, 1981).

Group I (non-exposed): Animals were injected with 2 doses of normal saline in an equivalent volume to the testing substances. The first was applied subcutaneously (s.c.) while the second one was injected intraperitoneally (i.p.) at the same time.

Group II (EA-sensitized): Animals were sensitized with 2 doses of EA suspension (Prolabo, France) in a concentration of 40 mg/100 gm body weight (s.c. and i.p. injections at the same time).

Group III (CDE-dosed): This group was treated with two equal doses of CDE, each dose contained the equivalent of 10 mg cotton dust powder/100 gm body weight (s.c. and i.p. injections at the same time).

Three weeks later all animals were killed by cervical dislocation and subjected to the experimental protocols described below. Animals in all groups were pretreated i.p. with different arachidonic acid metabolite synthesis inhibitors two hours before their killing. Doses were chosen on the basis of previous studies and according to preliminary studies which had been performed in our laboratory.

Indomethacin (Sigma, U.S.A.), a cyclooxygenase inhibitor (1 mg/kg) dissolved in 0.4% sodium bicarbonate.

Nordihydroguaiaretic acid (NDGA, Sigma, U.S.A.), a 5-lipoxygenase inhibitor (9 mg/kg) dissolved in 0.05% ethanol.

Quinacrine dihydrochloride (Mepacrine, BDH, England), a phospholipase A_2 inhibitor (8.1 mg/kg) dissolved in saline.

Imidazole (Sigma, U.S.a.), a thromboxane synthetase inhibitor (200 mg/kg) dissolved in saline.

Effect of CDE and EA on PPP of Guinea Pig Perfused Lung

After killing, lungs and trachea were dissected from the thorax and perfusion was immediately commenced via a tracheal cannula. The perfusion fluid was Krebs solution gassed with 95% O_2 and 5% CO_2, pH 7.4 and maintained at 37°C. Lungs were placed in a heated water-jacket (37°) and perfusion flow rate through the lungs was kept constant at a rate of 5.5 ml/min by means of a peristaltic pump (LKB, Sweden). Perfusion fluid passed through the alveolar walls and the perfusate was collected for determination pf PGs and LT-like substances. Changes in intralumenal PPP were continuously monitored proximal to the trachea by means of a mercury manometer.

Cotton dust extract (equivalent to 50 mg powder) and EA (10 mg) suspensions were introduced into the perfusion fluid in 1 ml volume via an injection port proximal to the trachea.

Biological Assay of Leukotrienes-Like Substances on Guinea Pig Ileum

A 3 cm piece of terminal ileum of the guinea pig was mounted in an organ bath (10 ml) containing aerated Tyrode solution at 37°C. Contractions elicited by various agonist (e.g. histamine dihydrochloride, BDH, England) were recorded by isotonic transducer (Washington T_2) connected to a chart recorder (Washington 400 MD_2C Oscillograph). When responses of the preparation to a submaximal dose of histamine had become constant the bathing fluid was changed to Tyrode solution containing atropine sulphate (3.4 mg/ml; BDH, England), mepyramine maleate (0.34 mg/ml; May & Baker, Germany) and methysergide bimaleate (0.1 mg/ml; Sandoz, Switzerland). These antagonists were present in concentrations sufficient to block the actions of maximally-effective concentration of acetylcholine, histamine and 5-hydroxytryptamine (5-HT), respectively. This preparation was then employed to assay LT-like substances in the perfusate. The specificity of the responses was checked by their sensitivity to the leukotriene antagonist, sodium 7-3-(4 acetyl-3-hydroxy-2-propylphenoxy)-2-hydroxypropoxy-4-oxo-8-propyl-4H-1-benzopyran-2-carboxylate (FPL $_{55712}$, 0.1 mg/ml, Fisons, England). The response was measured in millivolts and then converted to units. Each unit is equivalent to the response produced by 5 ng/ml histamine base (Chakravarty, 1959).

Biological Assay of Prostaglandin-Like Substances

Rat fundus stomach strip (4-5 cm long) was mounted in an organ both (10 ml) containing Krebs saline solution at 37°C gassed with 95% O_2 and 5% CO_2 (V/V). Changes in tension of the preparation were recorded isometrically by a transducer (Cynamometer UF1) connected to a chart recorder (Washington 400 MD_2 C Oscillograph). When responses of the preparation to a submaximal dose of PGE_2 had become constant, the bathing fluid was changed to a Krebs solution containing atropine sulphate (3.4 mg/ml, BDH, England), mepyramine maleate (0.34 mg/ml, May & Baker, Germany) and methysergide bimaleate (0.1 mg/ml, Sandoz, Switzerland) and FPL $_{55712}$ (0.1 mg/ml). These antagonists were present in concentrations sufficient to block the actions of maximally effective concentrations of acetylcholine, histamine, 5-HT and LTs respectively. This preparation was then employed to assay PGs in the perfusate (Al-Ubaidi and Bakhle, 1980).

Cohort 2
This group of animals was sub-divided into four test groups and a control group (five animals each) to study the effect of CDE on skin vascular permeability.

Group I (control group): Animals received normal saline intradermally (i.d.) in a volume equivalent to that used for CDE or EA.

Group II (EA-non-sensitized group): Guinea pigs were injected i.d. with EA in a dose of 40 mg/100 gm body weight without previous exposure to the sensitizing agent.

Group III (EA-sensitized group): This group was initially sensitized with two equal doses of EA suspension in a concentration of 40 mg/100 gm body weight. The first dose was applied s.c. and the second was applied i.p. at the same time. Three weeks later, the sensitized animals were injected i.d. with EA in the same dose.

Group IV (CDE-non-exposed group): Guinea pigs were directly injected i.d. with CDE in a dose equivalent to 10 mg powder/100 gm body weight.

Group V (CDE-exposed group): Animals were initially treated with two equal doses of CDE; each was equivalent to 10 mg cotton dust powder/100 gm body weight (s.c. and i.p. injections at the same time). Three weeks later, animals were treated i.d. with the extract at the same dose.

All groups of animals were pretreated i.p. with a single dose of the previously mentioned arachidonic acid metabolite synthesis inhibitors, two hours immediately before carrying out the experiment.

Determination of Vascular Permeability by Evans Blue Dye Method
Guinea pigs were anaesthetized with pentobarbitone sodium (60 mg/kg, i.p.). Animals were injected through the jugular vein with Evans blue dye (35 mg/kg; Aldrich, England). After 5 min, the test materials were injected i.d. in a dose of 40 mg/100 gm body weight for EA or the equivalent of 10 mg powder/100 gm body weight of CDE at a mechanically shaved skin area free of abrasion on the dorsal aspect of the animal. Thirty minutes later the injected skin site was excised, the dye extracted and measured spectrosphotometrically at 620 nm according to the previously-described method (Katayama et al., 1978).

$$\% \text{ Recovery} = \frac{\text{Concentration (mg/gm tissue}}{\text{Amount of dye injected}} \times 100$$

Results

Standardization of the Response
Acetyl choline (Sigma, U.S.A.) was employed as a reference bronchoconstrictor agent for each lung preparation. The rise of PPP to a supramaximally-effective dose of acetylcholine was established. All other bronchial responses were expressed as a percentage of this response to reduce the biological variation between animals.

Effect of CDE and EA on PPP of guinea pig
The addition of either CDE or EA to the perfusion fluid produced an increase in PPP in the previously non-exposed perfused lung. However, he same dose of both agents induced a more significant increase in PPP of the lungs from animals previously exposed to CDE or EA (Table 1).

The use of indomethacin (1 mg/kg) significantly reduced the EA-induced increase in PPP of previously non-exposed lungs ($P < 0.001$) and had no marked effect on that produced by CDE. Similarly, NDGA (9 mg/kg) and quinacrine (8.1 mg/kg) were able to reduce the increase of PPP induced by either CDE or EA. It was found that treatment of animals with quinacrine produced the highest reduction in PPP compared with the non-treated group. On the other hand, imidazole (200 mg/kg) had no significant effect on the rise in PPP induced by either agent (Table 1).

Concerning the sensitization of guinea pigs with EA or the previous exposure to CDE (similar to EA sensitization procedures), the increase in PPP in both groups was found to be significantly reduced by the previous administration of indomethacin, NDGA and quinacrine (Table 1). Imidazole did not induce any significant alteration in PPP induced by CDE or EA.

Effect of CDE and EA on the Amount of LT-Like Substances
It was found that CDE and EA were able to release higher amounts of LT-like substances in all groups of animals (either previously or on-previously-exposed to these agents) however, these levels were markedly increased in case of previous exposure to EA or CDE (Table 2). The use of arachidonic acid metabolite synthesis inhibitors revealed that indomethacin was the only inhibitor able to increase the amount of LT-like substances in the perfusate compared with the non-treated group (Table 2). The use of NDGA and quinacrine completely inhibited such activity. Imidazode had no significant effect on the amounts of LT-like-substances detected in the perfusage.

Effect of CDE and EA on the Amount of Prostaglandin-Like Substances
It was found that exposure of the lung (from guinea pigs either previously or non-previously exposed to EA or CDE) to either CDE or EA released significant amounts of PG-like substances in the perfusage (Table 3). These substances were significantly increased on pre-treatment of animals with NDGA (9 mg/kg) compared with the non-treated group. Imidazole treatment did not significantly change the amount of PG-like substances. On the other hand, indomethacin and quinacrine completely inhibited the release of these substances in the perfusate (Table 3).

Effect of CDE and EA on Vascular Permeability

Intradermal injection of guinea pigs either with CDE or EA resulted in a statistically significant increase in vascular permeability at the injection site compared to the control group ($P < 0.001$, Fig. 1). The previous exposure of animals to either CDE or EA produced a significant increase in the vascular permeability after three weeks of exposure compared to their respective non-exposed controls. In all groups of animals (either previously or non-previously to EA & CDE), it was found that treatment with quinacrine (8.1 mg/kg), NDGA (9 mg/kg) or indomethacin (1 mg/kg) produced a significant decrease in vascular permeability compared to non-treated animals. The results also indicated that quinacrine was the most effective agent. Imidazole (200 mg/kg) was not significantly effective in preventing the changes in vascular peremeability (Fig. 1).

Discussion

Inhalation of cotton dust leads to the development of the occupational lung disease, byssinosis in exposed workers. The actual pathogenesis of this disease is still not clear but the ability of cotton dust or its extract to release some inflammatory mediators may play an important role in the etiology of byssinosis (Bouhuys and Van de Woestigne, 1970).

The present work was carried out in part to study whether cotton dust produces its characteristic pulmonary effects due to a sensitizing activity following exposure or a direct effect without sensitization. Egg albumin as a known sensitizing agent was used as a standard.

The effect of CDE on the release of arachidonic acid metabolites from lung tissues was examined by using a perfused lung system where changes in gross airway reactivity may be measured following antigen provocation. In this system, the elevation of intraluminal perfusion pressure (PPP) following antigen challenge is due to airway constriction (Clay et al., 1985). The relationship between PPP and mediator release upon challenge with either CDE or EA was investigated.

Acetylcholine and histamine are known bronchoconstricting agents often used in inhalation challenges in man to evalute non-allergic bronchial excitability (Boushey et al., 1980). These agents provided a standard response to which responses by other constricting agents (such as CDE) were compared.

The effects of CDE as a possible sensitizing agent was also studied using the cutaneous anaphylaxis method as a marker for such an effect in the guinea pig. This was achieved by applying the cutaneous anaphylactic reaction in the in-situ skin of guinea pigs using the Evans blue dye extraction method. This method has been found to produce more precise quantitative evaluation than other methods which involve measuring the diameter of the extent of extravasated dye (Katayama et al., 1978). The method is generally used as in index of capillary permeability measurement because of its low toxicity and its stability to alkali and heating during its extraction.

It was found that challenege of animals with CDE or EA produced a significant increase in the PPP in addition to the release of a consderable amounts of LTs and PG-like substances in the lung perfusate. Such changes were observed in all groups of animals, both naive and previously exposed to CDE or EA. The release of LT- and PG-like materials in the perfusate could be responsible for this elevation in the PPP since thse arachidonic acid metabolites have proved to be highly potent contractile agents for parenchymal lung strips (Piccentini and Kaliner, 1991; Ohickey et al., 1991). This explanation is supported by the use of arachidonic acid metabolite inhibitors such as indomethacin, NDGA and quinacrine which were able to reduce the increase in PPP induced by CDE or EA. The previous studies have shown that the actively sensitized guinea pigs lung released LT-like substances upon challenge with specific antigen (Brocklehurst, 1960). These LTs were found to play a role in the pathogenesis of aiway hyper-responsiveness of lung disease such as asthma (Ohickey et al., 1991).

In this study, use of the EA sensitization technique was applied to the cotton dust to find out the possibility of antibody production following the exposure to CDE. The previous exposure to CDE produced a significant increase in PPP and the amount of arachidonic acid metabolites released in the lung perfusate compared to the non-previously exposed group. However, the previous exposure was not an essential factor for the release of these mediators, since the non-previously exposed lung releases substantial amounts of these contractile agents upon challenge with CDE and EA. It is not clear whether such effect is a speciific reaction for cotton dust and its extract through production of speciific antibodies. In general, CDE followed a similar pattern of lung responses to that produced by the standard antigenic agent, EA.

The skin reaction of CDE or EA using Evans blue eye as a markder produced a biphasic response in which there were early edematous wheel and flare reactions (similar to those observed with histamine) followed by an exudative phase characterized by a cellular infiltrate migrating from perivascular areas (Solley et al., 1976). The previous exposure of animals to either CDE or EA was not essential for the appearance of this response. However, the degree of vascular permeiablity was dependent on this previous exposure. The previously exposed animals to CDE or EA showed a higher degree of vascular permeability compared to the non-exposed group. These results were found to be similar to those from the lung perfusion technique concerning the effect of previous exposure. However, the skin reaction in naive individuals never before exposed to cotton dust suggests that the response is a non-specific

inflammlatory skin reaction and not conditioned by previous exposure to a specific antigen (Schachter et al., 1985).

Indomethacin, which is a selective cyclooxygenase inhibitor was able to reduce both PPP and cutaenous permeability in previously-exposed animals to CDE and EA. The reduction of PPP may be attributed to the inhibition of prostaglandin synthesis responsible for bronchoconstriction in guinea pig lung (Hitchicock, 1980). However, the mechanism by which PG induces its effect on the skin is thought to be through direct and indirect ways. The direct effect is on the vascular bed while the indirect effect seems to include a facilitation of axon reflex-mediated vasodilatation (Wallengeren and Hakanson, 1992).

Treatment of animals with indomethacin also released higher amounts of LT-like materials in the lung perfusate on comparison with the non-treated group. This may be due to the increased availability of arachidonic acid to be metabolized into lipoxygenase product (Burka and Saad, 1984). The latter include LT-like substances which are well recognised as bronchoconstrictor agents. Such metabolties constrict airway and vascular smooth muscle from the lungs of several species (Piper, 1984). In addition, these leukotrienes have been found to be important mediators of allergic and anaphylactic reactions (Henderson, 1991).

Treatment of animals with NDGA, a selective lipoxygenase inhibitor, produced a signficant reduction of CDE or EA-induced increase in PPP of guinea pigs. It was able to completely inhibit the release of LT-like materials from the perfused lungs of animals either previously exposed or non-exposed to CDE or EA. However, it caused an increase in the amounts of PG-like substances released in the lung perfusate. This effect is probably due to shifting the arachidonic acid metabolism down the cyclooxygenase pathway (Burka and Saad, 1984).

The inability of NDGA to produce a significant increase in PG-like substances released from the previously CDE-exposed lungs may be attributed either to the selective activity of CDE in releasing Lts to a greater extent than PGs (El-Mahdy and Nicholls, 1986) or to the small amounts of PGs released originally by CDE in the perfusate (Bates and Nicholls, 1988).

In addition treatment of all groups of animals with NDGA resulted in a significant decrease in the cutaneous permeability upon challenging the animals with CDE or EA. Lts, including LTC_4 and LTD_4, when injected into guinea pig or rat skin were found to cause increase in the skin permeability due to plasma exudation (Higgs, 1986). This is additional to their involvement as highly active mediators in cutanenous anaphylactic reactions and allergic conditions (Agarwal et al., 1992). These results indicate the important role of lipoxygenase products in the genesis of inflammatory and allergic analphylactic reactions induced by CDE and EA respectively.

Inhibition of both cyclooxygene and lipoxygenase products by quinacrine was of interest to evalute the degree to which arachidonic acid metabolits are involved in the inflammtory response to the dust extract. The inhibition of phospholipse A_2 by quinacrine induced a marked decrease in PPP upon challenge with either CDE or EA. This reduction in the perfusion pressure was demonstrated in all groups of animals independently on the previous exposure to the inflammatory agent. This marked decrease is associated with complete inhibition of the release of both Lts and PG-like materials in the lung perfusate.

Quinacrine did not completely abolish the elevation in PPP induced by either CDE or EA despite the complete inhibition of arachidonic acid metabolites released into the lung perfusate. this may be attributed to the release of other bronchoconstricting agents such as bradykinin and histamine from the lung tissue on exposure to CDE or EA (Nicholls, 1962). Previous studies have shown that an endotoxic lipoplysaccharide isolated from Enterobacter agglomerans, a major contaminant of cotton dust, is a potent stimulator of tumor necrosis factor-a (TNT-a) release from mast cells (Ryan and Karol, 1991). It was postulated that TNF-a might be released from the lung following exposure to cotton dust producing the pulmonary inflammatory response (Ryan and Karol, 1991).

Quinacrine was also able to reduce (but not completely) the elevated cutaenous vascular permeability induced by CDE or EA. Previous investigators showed that human mast cells are a source of pre-formed TNF-a (Gordon and Galli, 1990) which can be released rapidly upon exposure to antigen. TNF-a induces neutrophil accumulation and neutrophil dependent plasma exudation in rabbit skin (Rampart et al., 1989). Other mediators such as histamine and kinins might also mimic the cutaneous response to CDE or EA through chemotactic activity as well as increasing the permeability of the microcircucation (Wallengren and Hakanson, 1992).

Thromboxane A_2 as one of the major arachidonic acid metabolites does not appear to play an important role in CDE or EA-induced inflammatory processes. Imidazole as a selective TXA_2 syntehsis inhibitor did not have any significant effect on the elevated PPP or cutaneous vascular permeability induced by either CDE or EA. Although the levels of TXA_2 were not measured in the perfusate, it is expected that no significant amount is released as TXA_2 is not readily released from pulmonary endothelial cells or macrophages (Johnson et al., 1981) and the major source of TXA_2 release in vivo is platelets (Mundie and Ainsworth, 1985).

Summary

In conclusion, the use of guinea pig perfused lung served as a reliable tool to investigate the effect of CDE on the

pulmonary tissue. This study has highlighted the role of arachidonic acid metabolites in mediating the inflammatory skin reactions and elevation of PPP. However, the application of the sensitization technique of EA to CDE did not answer clearly the question about the possibility at cotton dust (or its extract) acting as a sensitizing agent. Both CDE and EA released the inflammatory mediators independently on the previous exposure. However, the magnitude of the release was different. The previously-exposed animals released higher amounts of arachidonic acid metabolites compared to the non-reviously exposed groups. The role of other inflammatory mediators also needs to be considered. Finally, although these results may suggest that CDE shares some of the sensitzing activity of EA, a more focused antigen-antibody reaction study must be effected to confirm this view.

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Table 1. Effect of cotton dust extract (CDE) and egg albumin (EA) on pulmonary perfusion pressure (PPP) of guinea pig lung.

	Non-Exposed	EA-Sensitized		CDE-Exposed		
	EA	CDE	EA	CDE	EA	CDE
Non-Treated	32.5±1.8	21.9±1.2	59.3±1.8	37.6±1.1	48.1±1.5	32.06±1.1
Indomethacin (1mg/kg)	23.8±0.8**	19.1±0.91	44.9±1.5***	28.6±1.7***	37.6±1.9***	25.3±1.8**
NDGA (9mg/kg)	25.1±1.6*	14.1±0.7***	50.1±2.08*	26.5±0.8***	39.4±1.2**	16.8±1.1***
Quinacrine	19.1±1.1***	12.4±1.2***	32.2±1.5***	20.1±1.1***	28.1±1.1***	12.9±0.7***
Imidazole (200 mg/kg)	27.6±1.9	21.7±1.6	54.9±1.9	32.4±2.1	43.7±1.6	30.8±1.3

Results are expressed as percentage of the supramaximal increase in PPP induced by acetylcholine (mean ± SEM of 5 animals). CDE (equivalent to 50 mg powder) and EA (10 mg) suspensions were introduced into the perfusion fluid in 1 ml via an injection port proximal to the trachea.
Significant differences are shown as * P<0.05; ** P<0.01; *** P<0.001 when compared with their respective non-treated group.
NDGA: Nordihydroguaiaretic acid

Table 2. Effect of cotton dust extract (CDE) and egg albumin (EA) on the amount of leukotriene-like substances in the perfusate of isolated guinea pig lung.

	Non-Exposed	EA-Sensitized		CDE-Exposed		
	EA	CDE	EA	CDE	EA	CDE
Non-Treated	54.00±2.3	48.00±3.1	80.67±4.7	70.00±3.1	72.00±2.3	62.00±3.5
Indomethacin (1mg/kg)	64.7±2.9*	60.7±1.7**	113.3±4.8**	90.7±4.8**	87.3±3.5**	76.0±3.4*
NDGA (9mg/kg)	ND	ND	ND	ND	ND	ND
Quinacrine	ND	ND	ND	ND	ND	ND
Imidazole (200 mg/kg)	60.0±4.2	51.3±0.67	77.3±3.5	67.0±1.5	68.0±2.3	56.0±6.1

Results are expressed as units. Each unit is equivalent to the contraction produced by 5 ng/ml histamine base on guinea pig ileum (mean ± SEM of 5 animals). CDE (equivalent to 50 mg powder) and EA (10 mg) suspensions were introduced into the perfusion fluid in 1 ml via an injection port proximal to the trachea.
Significant differences are shown as * P<0.05; ** P<0.01 when compared with their respective non-treated group.
ND: Non-detectable
NDGA: Nordihydroguaiaretic acid

Table 3. Effect of cotton dust extract (CDE) and egg albumin (EA) on the amount of prostaglandin-like substances in the perfusate of isolated guinea pig lung.

	EA	CDE	EA	CDE	EA	CDE
Non-treated	34.5±1.2	29.0±2.1	56.7±2.4	45.0±3.5	44.0±2.3	36.7±1.81
Indomethacin (1mg/kg)	ND	ND	ND	ND	ND	ND
NDGA (9mg/kg)	48.3±2.6**	39.3±4.1	68.0±1.2**	58.7±1.8**	54.0±2.0*	47.0±2.9*
Quinacrine	ND	ND	ND	ND	ND	ND
Imidazole (200 mg/kg)	33.0±1.5	28.7±2.9	62.7±1.8	49.3±3.5	43.7±4.4	34.7±2.3

Results are expressed as ng/ml using PGE_2 as a standard on rat fundus strip (mean ± SEM of 5 animals). CDE (equivalent to 50 mg poweder) andEAr (10 mg) suspensions were introduced into the perfusion fluid in 1 ml via an injection port proximal to the trachea.
Significant differences are shown as * P<0.05; ** P<0.01 when compared with their respective non-treated group.
ND: Non-detectable
NDGA: Nordihydrogeaiaretic acid