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Protein content and patient skin test sensitivity of the pollen of *Argemone mexicana* on exposure to SO₂

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SHORT COMMUNICATION

Protein content and patient skin test sensitivity of the pollen of *Argemone mexicana* on exposure to SO₂

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Parui S., Mondal, A. K. & Mandal, S. 1998. Protein content and patient skin test sensitivity of the pollen of *Argemone mexicana* L. on exposure to SO₂. – Grana 37: 121–124. ISSN 0017–3134.

The allergenic potency of pollen has been reported to be increased when exposed to atmospheric pollutants like SO₂, NO₂, etc. The present investigation was undertaken to study the effect of SO₂ pollution on protein content as well as the allergenic potency of the pollen of *Argemone mexicana* L.

The pollen of this plant was fumigated with SO₂ at a concentration of 100 ppm for 24 hours, 48 hours and 72 hours and the soluble protein content and the electrophoretic profile were studied. SDS-PAGE revealed the appearance of a new protein band with the simultaneous disappearance of two other bands. Protein content showed a gradual decrease with the increase in time of exposure to SO₂. Skin-prick test were performed with the antigens of the normal as well as SO₂ fumigated pollen. The skin prick test sensitivity and percentage of patients showing positive response increased with time of fumigation.

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Airborne pollen grains which have long been proved to be a cause of respiratory allergic disorders, are at present considered to be the major source of morbidity for atopic patients (Kjellman 1993). Currently, there has been a lot of speculation and discussions on the reasons behind the general increase of allergic diseases. Many scientists have concluded from their studies that the environmental factors, especially air pollution are responsible for increasing allergenicity of pollen (Breiteneder & Scheiner 1991, Jilek et al. 1993).

One of the common gaseous air pollutants which originates from combustion of coal and petroleum is sulphur dioxide, which is known to be injurious to human health causing respiratory diseases. Concentrations as low as 1–5 ppm can affect conditioned nerve reflexes and also breathing (Santra et al. 1991). SO₂ when taken up by the leaves of plants through open stomata, dissolves in the aqueous phase of cell walls, the apoplast and then diffuses into the mesophyll cells, where it reacts with water to form sulfurous acid which inhibits photosynthesis in the chloroplasts (Kaiser et al. 1993). However SO₂ has a different effect on the pollen that remain suspended in the air after being released from their respective sources. SO₂ may change the degree of allergenicity of pollen by changing the kind and proportions of exinic mineral elements (D'Amato 1991, Solomon 1986). It also affects the morphology of the grains as well as their protein content and electrophoretic profile (Santra et al. 1991).

The plant of *Argemone mexicana* L. growing in waste areas, road sides and along the railway tracks, is a common weed of West Bengal, India including the industrial centres of Calcutta, Burdwan, Durgapur, etc. It flowers almost throughout the year and its pollen has been reported in the air and proved to be allergenically potent by several workers

(Acharya 1980, Mari Bhat & Rajasab 1992, Jain et al. 1992, Shivpuri 1980, Tilak 1989). The present investigation was undertaken to study the effect of SO₂ on the protein components and the allergen content of the pollen grains of *A. mexicana* by artificial fumigation of pollen with SO₂ in the laboratory.

MATERIALS AND METHODS

Pollen grains of *A. mexicana* collected in bulk from plants growing in and around Santiniketan, West Bengal, were sieved using different meshes (100, 200 and 300 µm) to remove the debris and microscopically analysed to assure 85% to 95% of pollen purity. One set of the sample was subjected to SO₂ fumigation by separately exposing to 100 ppm. of SO₂ for 24 hours, 48 hours and 72 hours in a glass chamber according to the method of Santra et al. (1991) and the other set was kept as control. Protein from the pollen sample of both sets was extracted in 0.2 M Tris HCl buffer, pH 7.4 at 4°C following the method of Singh et al. (1993) with slight modifications and the protein concentration in each extract was quantified by the method of Lowry et al. (1951). SDS-PAGE was done following the method of Laemmli (1970), using a 10% T mini-gel (8 × 7 cm gel). The gel was calibrated with marker proteins (M.W. range – 29 kDa to 205 kDa) obtained from Sigma Co., U.S.A. After electrophoresis, the gel was stained with 0.1% Coomassie Brilliant Blue R 250 and destained with methanol:acetic acid:water (4:1:5) mixture.

Pollen antigens were prepared from all the samples by the method of Sheldon et al. (1967) and the skin prick tests performed at Calcutta Medical Research Institute, Calcutta, India. Each patient was tested with 10 µl of the total extract diluted with 0.05 M Phosphate buffered saline, pH 7.4, to a concentration of 1 part by weight of protein in 500 parts by volume of the buffered saline. Negative and positive controls were also performed. The negative control was buffered saline and the positive control was histamine acid phosphate injection diluted with buffered saline to 1 µg of histamine acid phosphate. The skin reactions were read after 15 to

20 min from the start of the test and quantified on the basis of the wheel diameter and graded 1+ to 4+ (Shivpuri 1980).

RESULTS AND DISCUSSION

SO₂ fumigated pollen of *A. mexicana* revealed noticeable variations in the protein content as well as the electrophoretic protein profile. The results have been presented in Table I. The soluble protein content of the exposed pollen decreased with an increase in the exposure dose of SO₂. The protein content in the normal pollen grains was found to be 29.5% which decreased to 27.36% in the 72 hours fumigated pollen. The electrophoretic patterns of the protein of SO₂ exposed pollen also showed variation as revealed by SDS-PAGE. The protein profile of the normal pollen showed 13 bands (Fig. 1) which were designated as AM1 to AM14 with their molecular mass ranging between 31 kDa and 198 kDa with another band having a molecular mass greater than 198 kDa (AM1). AM11 was absent in the normal protein fraction. However, the protein profile of SO₂ fumigated pollen showed the

complete disappearance of AM1 (>198 kDa) and AM2 (198 kDa) fractions, with the appearance of a new soluble protein fraction (AM11) having a molecular weight of

Table I. Protein content (%) of the pollen of *Argemone mexicana* fumigated at different time exposure of SO₂.

	SO ₂			
Control	24 hrs	48 hrs	72 hrs	
29.50	29.31	28.20	27.36	

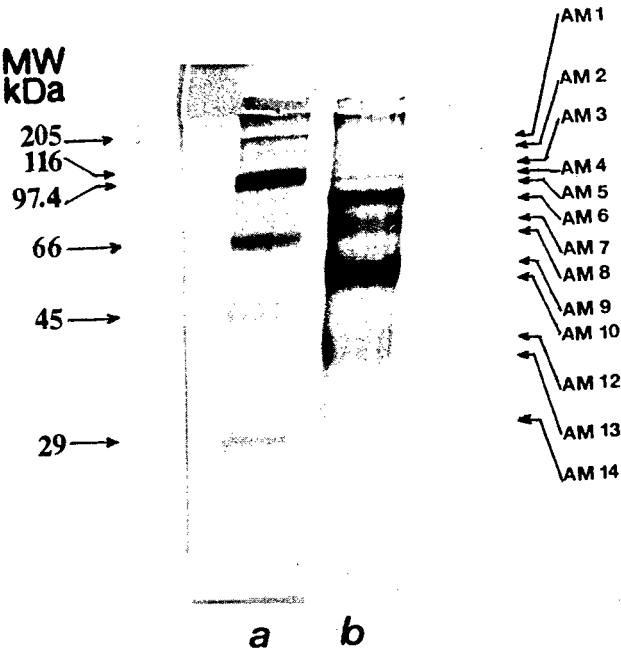


Fig. 1. SDS-PAGE pattern of the antigenic extract of the pollen of *A. mexicana* before SO₂ fumigation. (a) represent the marker mixture (M.W. Range 29 kDa to 205 kDa); (b) contains 46 µg of crude antigen showing 13 bands; AM1 (>198 kDa), AM2 (198 kDa), AM3 (149 kDa), AM4 (138 kDa), AM5 (95 kDa), AM6 (85 kDa), AM7 (77 kDa), AM8 (66 kDa), AM9 (59 kDa), AM10 (57 kDa), AM12 (40 kDa), AM13 (39 kDa) and AM14 (31 kDa) with the absence of AM11.

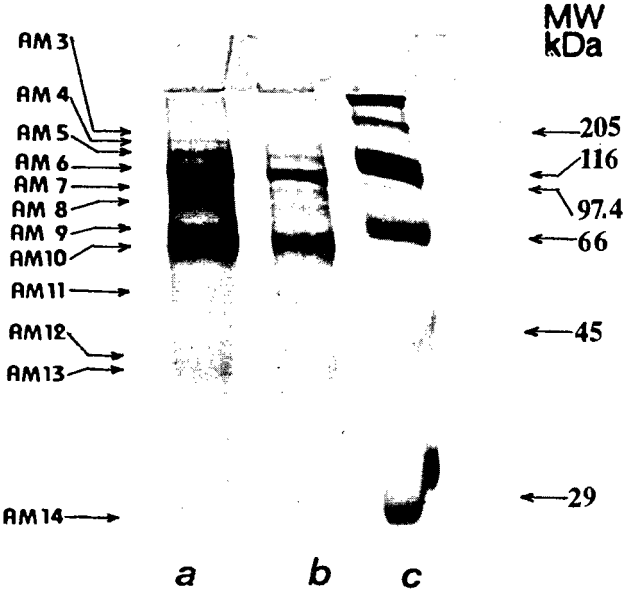


Fig. 2. SDS-PAGE pattern of the antigenic extract of the pollen of *A. mexicana* after SO₂ fumigation for 72 hours showing the absence of AM1 (>198 kDa) and AM2 (198 kDa) and the appearance of a new band AM11 (53 kDa). (a&b) contain 54 µg and 46 µg, respectively, of crude antigen; (c) represents marker mixture.

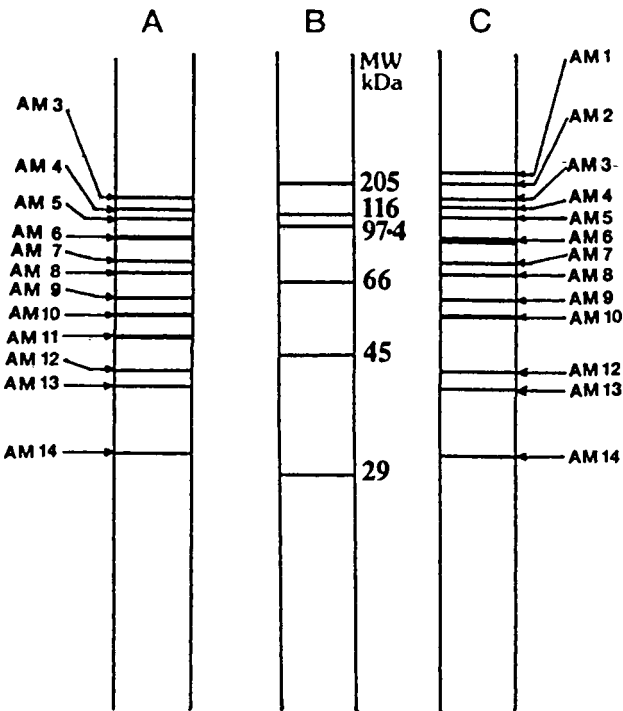


Fig. 3. Diagrammatic representation of the comparative SDS-PAGE pattern of the pollen extract of *A. mexicana*. (a) Pollen extract after SO₂ fumigation for 72 hours; (b) MW marker; (c) pollen extract before SO₂ fumigation.

Table II. Results of skin tests against the antigen of the pollen of *Argemone mexicana* fumigated at different time exposure of SO₂ in patients of nasobronchial allergy and controls (normal persons).

Diameter of erythema (mm)	0	Reactivity	0
	1-5		1+
	6-10		2+
	11-15		3+

Antigen tested	Patients						Normal			
	Total number of tests	Negative %	1+ %	2+ %	3+ %	4+ %	Total number of tests	Negative %	1+ %	2+ %
1) Control	43	56.0	28.2	14.0	1.8	0.0	20	95	5	0
2) SO ₂ fumigated										
a) 24 hrs	43	53.6	29.1	15.5	1.8	0.0	24	92	8	0
b) 48 hrs	43	48.4	32.2	17.0	2.1	0.3	20	89	11	0
c) 72 hrs	43	46.2	36.8	14.0	2.2	0.8	20	83	15	0

53 kDa (Figs. 2&3). Similar results have earlier been reported by Santra et al. (1991) in *Datura metel* and *Cocos nucifera*.

Skin prick tests revealed the pollen of *A. mexicana* to be allergenically potent. 43 patients were tested, of whom 44% showed positive response to the antigen of *A. mexicana* (Table II). 28.2% of the patients showed 1+ reaction, 14.0% showed 2+ reaction and 3+ reaction was given by just 1.8% of the total sensitive patients. None of the patients showed 4+ reaction. Exposure to SO₂ showed an increase in the number of patients showing positive response to the pollen of this plant. 46.4%, 51.6% and 53.8% patients were found to be sensitive to the antigenic extracts of 24 hours, 48 hours and 72 hours fumigated pollen respectively. There were also patients who showed 4+ reaction in case of pollen exposed to SO₂ for 48 hours and 72 hours. Among the tests conducted with the total extract on 20 normal persons, one showed 1+ reaction with the extract of unfumigated pollen. This incidence of positivity could be due to irritants present in the extracts or may be due to the contaminating constituents unrelated to the actual allergen to which a person has been actually sensitized, as the total pollen extracts are heterogeneous crude extracts containing proteins, enzymes, lectins, glycoproteins, lipoproteins, etc. and may contain the relevant allergen in minute and irreproducible amounts as pollen allergens generally comprise 0.5 to 1.0% of the total extractable proteins (Stanley and Linskens, 1974). These contaminating constituents may lead to the induction of IgE antibodies with new specificities (Mohapatra 1994). However, extracts of SO₂ fumigated pollen showed an increase in the number of normal people showing positive reaction. 15% of the patients showed 1+ reaction with 72 hours fumigated pollen extract. This increase in the incidence of positivity in both the normal persons and the patients could be explained by the fact that the appearance of the new protein band AM11 could be responsible for inducing positive reaction with new specificities.

CONCLUSION

The rapid industrialization in most of the major cities of our country together with the heavy vehicular exhausts makes

it very difficult to properly diagnose the causative agent of respiratory disorders. Grimfeld (1988) had reported increased asthma in children due to SO₂, cigarette smoke and air pollution, while Price (1990) had suggested that air contaminants such as SO₂ and other chemical pollutants are responsible for developing asthma in industrial regions. Thus for patients suffering from respiratory disorders due to airborne pollen, the SO₂ pollution could be responsible for the increase in sensitivity as the allergenicity of a pollen increases considerably with exposure to atmospheric SO₂, as has been observed in the present investigation.

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