SHORT COMMUNICATION

EFFECTS OF CHRYSOTILE ASBESTOS EXPOSURE ON CERTAIN BIOCHEMICAL PARAMETERS OF *LEMNA GIBBA*

A.K. TRIVEDI*, I. AHMAD AND M. ASHQUIN

Fibre Toxicology Division, Indian Institute of Toxicology Research, M.G. Marg, P.B. No. 80, Lucknow-226 001, India

Received on 28 Oct., 2009, Revised on 5 Feb., 2010

SUMMARY

Asbestos and its toxic potential on animal and human system has been extensively documented but meager information is available regarding its effects on plant model. Previously, we have shown that chrysotile exposure exerts significant alterations in growth, physiological and biochemical parameters of an aquatic macrophyte, duckweed (*Lemna gibba.*). In the present study *Lemna gibba* plants were exposed to four concentrations (0.5, 1.0, 2.0, and 5.0 µg ml^{-1}) of chrysotile asbestos under laboratory conditions and alterations in the level of certain oxidative stress enzymes were evaluated at post exposure day 28 in order to assess changes in their level as suitable biomarkers of chrysotile contamination because changes in their level will affect growth, development and physiology at molecular level. Chrysotile exposure caused an increase in the lipoxygenase, peroxidase, glutathione reductase, glutathione S-transferase and monodehydroascorbate reductase activity and a decrease in dehydroascorbate reductase activity. These alterations might be considered as biomarkers of exposure to unsafe environment because oxidative stress due to environmental constraints renders such changes in plants to overcome oxidative stress and tolerate unsafe environment.

Key words: **Chrysotile, duckweed, *Lemna gibba,* oxidative stress, reactive Oxygen Species (ROS) and toxicity.**

In India, several states have many asbestos industries out of which 60% are in operation and production is about 2000 tones per month (Ramanathan and Subramanian 2001). Chrysotile is emitted from both natural and industrial sources. It exhibits significant solubility in aqueous neutral or acidic environments. Chrysotile and its associated serpentine minerals chemically degrade at the surface, this in turn produces measurable effects on plant growth. In natural condition chrysotile fibers can be transported by wind and water (IPCS 1998). Therefore, evaluation of their effects on plants growing in the nearby ecosystem can address many questions about physiological impact of asbestos.

Various health effects in man and laboratory mammals have been documented due to chrysotile fiber exposure (Hauptman *et al.* 2002). However, the potential ecological impact of this material has largely been ignored (NIPHEP 1989). Chrysotile fibers carry a positive surface charge at pH < 11.8 (Speil and Leinweber 1969). These charged fibers presumably would be attracted to negatively charged protein groups

*Corresponding author, E-mail: ajaykumartrivedi@gmail.com; Present address: National Bureau of Plant Genetic Resources, Regional Station, Bhowali-263 132, Niglat, Distt.-Nainital (Uttarakhand), India

in cell membrane. The chrysotile fiber would then be surrounded by proteins and submerge into the cell. This series of events could be a mechanism by which fibers could gain entry into the cell (Harington *et al*. 1975).

Duckweed (*Lemna gibba*) is an important food species for aquatic herbivores, a good dietary supplement and nutrient source (Oron *et al*. 1985) for humans, livestock, fish and is used as a good fertilizer supplement and also as an indicator of water pollution. It is widely recommended for aquatic toxicity studies (EPA 1985) because of its cast effectiveness and reliability (Taraldsen and Norberg-King 1990). The selection of exposure concentration is based on the range of fibers found during monitoring (Trivedi *et al*. 2007). Present study reports the toxic effects of chrysotile asbestos on certain enzymes related to oxidative stress.

To study toxic effects of chrysotile asbestos on *L. gibba*, plants were collected from the natural habitat in an aquatic body, washed axenically and maintained in Hoagland medium in the laboratory under light and dark period of 16/8 day/night and controlled humidity (60%). The young plants of the third generation were transferred to sterilized petri dishes and used for experiment. Chrysotile fibers of size <30 μm were used in the study. Adding 0.5 g, 1.0 g, 2.0 g, and 5.0 g chrysotile four suspensions of chrysotile fibers were prepared separately, in 1.0 litre of double distilled water with constant stirring. Five micro litre suspension was applied per frond twice a week. These plants were cultured in a medium containing 0.1 g per litre chrysotile in order to reproduce conditions similar to field, where some chrysotile fibers enter in the aquatic system with drainage water as well as falls on the fronds through air. Control plants were cultured in nutrient medium without chrysotile fiber. Experiments were carried out for test duration of 28 day and response parameters were determined immediately after the exposure period.

At the end of the exposure period the fronds were washed with double distilled water, patted dry between two layers of filter paper and weighed. They were homogenized in 1.5 ml of cold 5% (w/v) metaphosphoric acid with a potter homogenizer (Servodyne mixer head, Cole Parmer, Niles, IL, USA). The homogenate was then centrifuged for 30 min at 19000 x g and the clear supernatant was used for the determination of different enzymes. Lipoxygenase (LOX) (EC 1.13.11.12), Peroxidase (POD) (EC 1.11.1.7) and Glutathione Reductase (GR) activity (EC 1.6.4.2) was measured by the methods of Bostock *et al*. (1992), Cordemener *et al*. (1991) and Smith *et al*. (1988), respectively. Glutathione S-transferase (GT) activity (EC 2.5.1.18) was assayed as described by Drotar *et al*. (1985). Monodehydroascorbate Reductase (MDHAR) (EC 1.6.5.4) and Dehydroascorbate Reductase (DHAR) activity (EC 1.8.5.1) was assayed according to method given by Hossain *et al*. (1984) and Hossain and Asada (1984), respectively. Experiments were conducted in five replications. Data presented are mean of three independent experiments. Data for each parameter were evaluated for statistical significance using two-way analysis of variance (ANOVA) to compare the means considering duration of exposure and concentration as independent variables. The individual treatment between the two groups was assessed by computation of least significant difference taking 't' values for error d.f. at the 5% (*) and 1% (**) level of significance.

In natural condition plants are often exposed to various environmental stresses, which adversely affect growth, development as well as metabolism. At the whole-plant level, the effect of stress is usually perceived as a decrease in growth and metabolism, and is associated with alterations in activities of enzymes. Chrysotile exposure was found to significantly increase profiles of LOX activity in 2.0 μg frond^{-1} ($p<0.05$) and 5.0 μg frond^{-1} ($p<0.01$) chrysotile exposed plants (Fig. 1). The increase in LOX activity might be the reason of increased lipid peroxidation which is the symptom most easily ascribed to oxidative damage (Zhang and Kirkham 1996). As compared to control a sifgnificant increase in peroxidase activity was found in 2.0 μg frond^{-1} and 5.0 μg frond^{-1} ($p<0.01$) chrysotile exposed plants (Fig. 1). Peroxidase activity is related to the generation and use of ROS (Van Huystee, 1987). Plant peroxidases exist in a variety of isoforms that use different reductants and are located in different cellular compartments (Campa 1991). POD activity uses H_2O_2 as a substrate. The increase in specific activity of POD may be due to induction by H_2O_2 and or by decrease in total protein. A significant increase in GR activity was also recorded in 2.0 μg frond^{-1} ($p<0.05$) and 5.0 μg frond^{-1} ($p<0.01$)

Fig. 1. Changes in specific activity of Lipoxygenase (LOX), Peroxidase (POD) and Glutathione Reductase (GR). Values presented are means ± S.E. expressed as percent of respective controls. Control values were 1.05 ± 0.21 µmol, 2.28 ± 0.15 µmol and 0.071 ± 0.02 µmol substrate min^{-1} mg^{-1} protein for LOX, POD and GR, respectively on day 28. ($^{*}P<0.05$, $^{**}P<0.01$).

Fig. 2. Changes in specific activity of Glutathione S-transferase (GT), Monodehydroascorbate Reductase (MDHAR) and Dehydroascorbate Reductase (DHAR). Values presented are means ± S.E. expressed as percent of respective controls. Control values were 0.20 ± 0.02 µmol, 1.09 ±0.15 µmol and 0.15 ± 0.01 µmol substrate min^{-1} mg^{-1} protein for GT, MDHAR and DHAR, respectively on day 28. ($^{*}P<0.05$).

chrysotile exposed plants (Fig. 1). Previous study shows that antioxidant metabolism can be stimulated by increases in ROS, although basal developmental factors may also play a decisive role (Donahue *et al*. 1997). Increases in GR activity under stress of cold, high light, ozone and water has been already reported (Burke *et al*. 1985).

GT activity was found to be significantly increased in plants exposed to 2.0 µg $frond^{-1}$ and 5.0 µg $frond^{-1}$ ($p<0.05$) chrysotile (Fig. 2). GTs have been shown to be induced by a wide range of chemical agents as well as wounding, heavy metals, ethylene and ozone (Marrs 1996). Furthermore, ascorbate is regenerated either non enzymatically by electron transport chain or enzymatically (using MDHAR) from monodehydro ascorbic acid (MDHA) or from dehydro ascorbic acid (DHA) by the ascorbate-glutathione cycle. Two ascorbate recycling enzymes monodehydroascorbate reductase and dehydroascorbate reductase show increased oxidative stress induced activity (Knorzer *et al*. 1996). Significant increase in MDHAR activity was found in plants exposed to 2.0 µg $frond^{-1}$ and 5.0 µg $frond^{-1}$ ($p<0.05$) chrysotile (Fig. 2). On the contrary a significant decrease in dehydroascorbate reductase activity was noticed in plants exposed to 2.0 µg $frond^{-1}$ and 5.0 µg $frond^{-1}$ ($p<0.05$) chrysotile (Fig. 2) which can be interpreted as a peroxidative enzyme destruction or an inactivation of the enzyme, e.g., by suppression of gene expression through as yet unknown regulation (Knorzer *et al*. 1996).

In conclusion present findings suggest that chrysotile asbestos contamination causes oxidative stress induced alterations in enzyme system and in turn growth, development and physiology of plants to overcome the stress condition. Biochemical status of the cell, particularly activities of certain enzymes in *L. gibba* might serve as suitable biomarkers of exposure to environmental contamination of chrysotile asbestos and indicator of chrysotile asbestos mediated toxicity. Further studies are suggested in this direction to confirm the mechanism at molecular level as well as to validate their use under field conditions.

ACKNOWLEDGEMENT

Authors are thankful to the Director, Indian Institute of Toxicology Research, Lucknow for his keen interest in the study. Financial assistance from the Ministry of Environment and Forests (Govt. of India), New Delhi is also gratefully acknowledged.

REFERENCES

Bostock, R.M., Yamamoto, H., Choi, D., Ricker, K.E. and Ward, B.L. (1992). Rapid stimulation of 5-Lipoxygenase activity in potato by the fungal elicitor arachidonic acid. *Plant Physiol.* **100:** 1448-1456.

Burke, J.J., Gamble, P.E., Hatfield, J.L. and Quisenberry, J.E. (1985). Plant morphological and biochemical responses

to field water deficits. I. Responses to glutathione reductase activity and paraquat sensitivity. *Plant Physiol.* **79:** 415-419.

Cordemener, J., Booij, H., van der Zandt, H., van Engelen, F., van Kammen, A. and de vries, S. (1991). Tunicamycin-inhibited carrot somatic embryogenesis can be restored by secreted cationic peroxidase isozymes, *Planta* **184:** 478-486.

Donahue, J.L., Okpodu, C.M., Cramer, C.L., Grabau, E.A. and Alscher, R.G. (1997). Responses of antioxidants to paraquat in pea leaves. Relationships to resistance. *Plant Physiol.* **113:** 249-257.

Drotar, A., Phelbs, P. and Fall, R. (1985). Evidence for glutathione peroxidase activities in cultured plant cells. *Plant Sci.* **42:** 32-40.

EPA (1985). U.S. Environmental Protection Agency, Lemna acute toxicity test, *Fed. Regist.* **50:** 39331-39333.

Harington, J.S., Allison, A.C. and Badami, D.V. (1975). Mineral Fibers: Chemical, physico-chemical and biological properties. *Adv. Pharmacol. Chemother.* **12:** 291-402.

Hauptmann, M., Pohlabeln, H., Lubin, J.H., Jockel, K.H., Ahrens, W., Bruske-Hohlfeld, I., Wichman, H.E. (2002). The exposure-time-response relationship between occupational asbestos exposure and lung cancer in two German case-control studies. *Am. J. Ind. Med.* **41:** 89-97.

Hossain, M.A. and Asada, K. (1984). Purification of dehydroascorbate reductase from spinach and its characterization as a thiol enzyme. *Plant Cell Physiol.* **25:** 85-92.

Hossain, M.A., Nakano, Y. and Asada, K. (1984). Monodehydroascorbate reductase in spinach chloroplasts and its participation in regeneration of ascorbate for scavenging hydrogen peroxide. *Plant Cell Physiol.* **25:** 385-395.

IPCS (1998). Environmental Health Criteria 203, Chrysotile Asbestos, Geneva, World Health Organization, International Programme on Chemical Safety, p 29.

Knorzer, O.C., Durner, J. and Boger, P. (1996). Alterations in the antioxidative system of suspension-cultured soybean cells (*Glycine max*) induced by oxidative stress. *Physiol. Plant.* **97:** 388-396.

Marrs, K.A. (1996). The function and regulation of glutathione *S*-transferases in plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **47:** 127-158.

NIPHEP (1989). National Institute for Public Health and Environmental Protection, The Netherlands. Report No. 758473013.

Oron, G., Wildschut, L.R. and Porath, D. (1985). Waste water recycling by duckwed for protein production and effluent renovation. *Water Sci. Technol.* **17:** 803-817.

Ramanathan, A.L. and Subramanian, V. (2001). Present status of asbestos mining and related health problems in India – A survey. *Ind. Health* **39:** 309-315.

Smith, I.K., Vierhelle, T.L. and Thorne, C.A. (1988). Assay of glutathione reductase in crude tissue homogenates using 5,5'-dithiobis (2 -nitrobenzoic acid). *Anal. Biochem.* **175:** 408-413.

Speil, S., Leinweber, J.P. (1969). Asbestos minerals in modern technology. *Environ Res.* **2:** 166-208.

Taraldsen, J.E. and Norberg-King, T.J. (1990). New method for determining effluent toxicity using duckweed (*Lemna minor*). *Environ. Toxicol. Chem.* **9:** 761-7676.

Trivedi, A.K., Ahmad, I., Musthapa, M.S. and Ansari, F.A. (2007). Environmental contamination of chrysotile asbestos and its toxic effects on antioxidative system of duckweed (*Lemna gibba*). *Arch. Environ. Contam. Toxicol.* **52:** 355-362.

Van Huystee, R.B. (1987). Some molecular aspects of plant peroxidase biosynthetic studies. *Annu. Rev. Plant Physiol.* **38:** 205-219.

Zhang, J. and Kirkham, M.B. (1996). Lipid peroxidation in sorghum and sunflower seedlings as affected by ascorbic acid, benzoic acid, and propyl gallate. *J. Plant Physiol.* **149:** 489-493.