

Protective role of ashwagandha in cadmium-induced hepatotoxicity and nephrotoxicity in male mouse

Withania somnifera (ashwagandha) of family Solanaceae is a sub-tropical undershrub. It is highly esteemed as a rasayana drug in Ayurveda. Its root extract is used as tonic, alterative, aphrodisiac, narcotic, diuretic, in emaciation of children, senile debility and in rheumatism¹. Its psychotropic, antitumour, cardiotoxic and respiratory stimulating effects have also been reported²⁻⁷. Despite the fact that ashwagandha is an important medicinal plant, its efficacy in relation to the regulation of cadmium toxicity, if any, was not studied earlier.

Cadmium is a well-known toxicant. The toxic effects of this metal in liver and kidney tissues of mammals have already been reported⁸. Other than some chelating agents^{9,10}, studies on the regulation of its toxicity have been restricted to a few antioxidants such as glutathione (GSH), selenium and tocopherol¹¹. We attempt here to reveal the protective effects of ashwagandha, if any, on the cadmium-induced toxicity in liver and kidney tissues of mouse.

Ashwagandha root spray dried powder was supplied by Kisalaya Pharmaceuticals Pvt Ltd, Indore, India. The dried powder was an alcoholic extract with 1.75% withanolides. The dried powder was used to prepare the aqueous extract of 40 mg/0.1 ml concentration for the treatment. 28 adult healthy colony bred Swiss male mice (approx. 3 month) weighing 30–35 g were maintained at constant temperature ($27 \pm 1^\circ\text{C}$) and light (14 L:10 D) controlled room with the provision of food (Hindustan Lever Ltd, India) and water *ad libitum*. Seven days after acclimatization, the mice were divided into four groups of seven each. Group I received 0.1 ml of normal saline per day, and served as control. Groups II, III and IV were treated with cadmium chloride (CdCl_2), CdCl_2 + ashwagandha root extract and ashwagandha extract alone, respectively. While the dose of CdCl_2 was 1.0 mg/day/animal^{12,13}, that of Ashwagandha was 1.14 g/kg body wt/day⁷. The experiment was continued for 20 days. On the last day, the animals were sacrificed, liver and kidney of each

animal were removed, cleaned twice in phosphate buffered saline (pH 7.4) and immediately processed for biochemical estimations. Lipid peroxidation (LPO) was estimated by the method of Ohkawa *et al.*¹⁴. The liver and kidney were homogenized (10% wt/vol) in ice cold phosphate buffer (0.1 M, pH 7.4) using Potter–Elvehjem teflon homogenizer and the homogenate was centrifuged at 2000 g at 4°C for 30 min. The amount of malondialdehyde (MDA) formed was measured by taking the absorbance at 532 nm using Shimadzu UV-160 A spectrophotometer (Japan). LPO was expressed as the amount of MDA formed (nM) per hour per mg protein. Protein was estimated by the method

of Lowry *et al.*¹⁵. SOD activity was determined by the method of Marklund and Marklund¹⁶, and CAT activity by the method of Aebi¹⁷.

Data have been finally expressed as the mean \pm standard error of the mean (SEM). For statistical evaluation, analysis of variance (ANOVA) and Student's *t* test were used¹⁸.

The results are summarized in Figure 1. A decrease in LPO and an increase in SOD and CAT were observed in liver homogenate when ashwagandha alone was treated. In kidney also, LPO was significantly decreased following ashwagandha treatment. On the other hand, a significant increase in the LPO in liver and kidney tissues was found

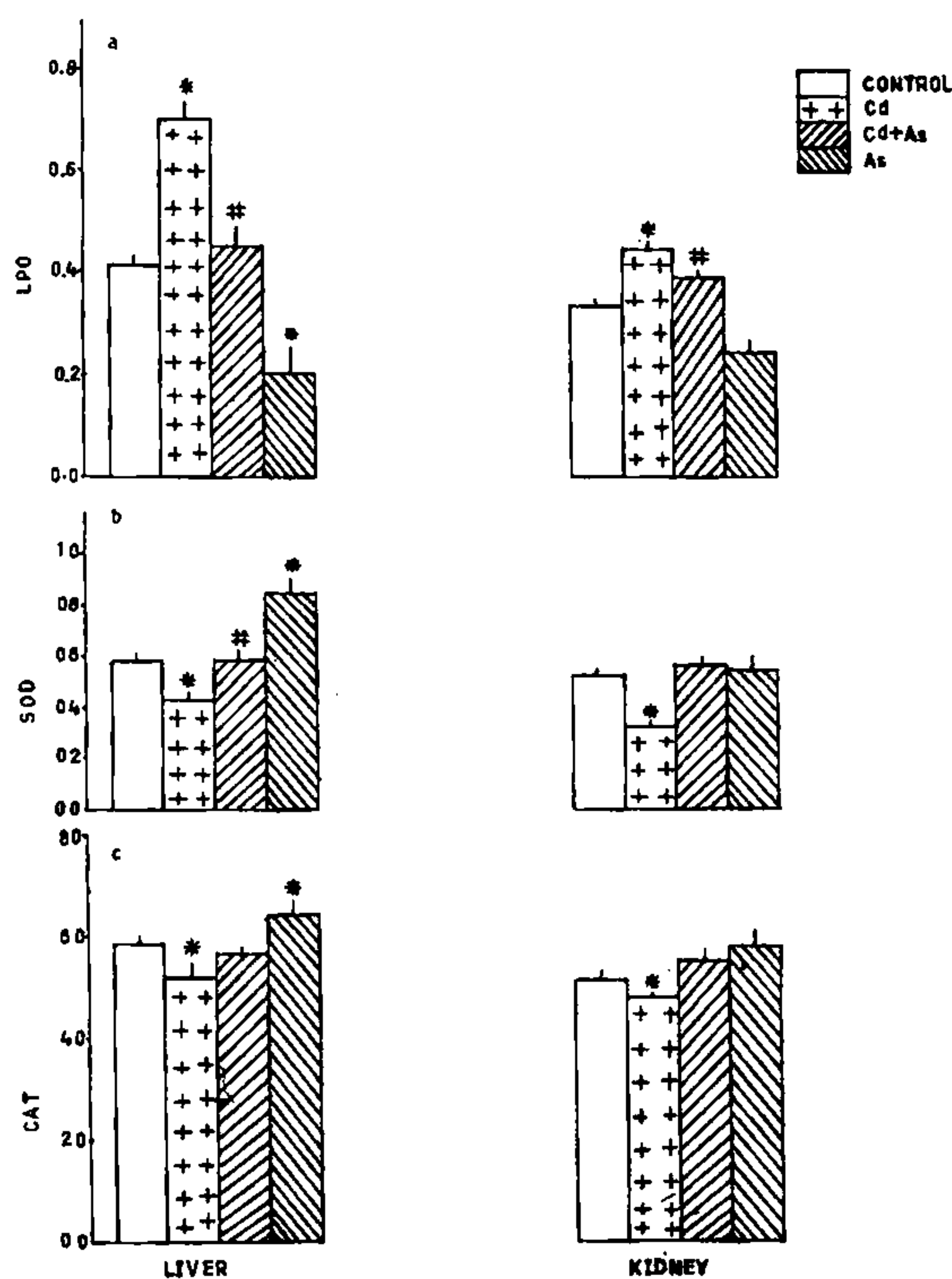


Figure 1. Effects of ashwagandha root extract (As, $1.14 \text{ g kg}^{-1} \text{ day}^{-1}$) for 20 days on cadmium-induced hepatic and renal LPO (nM MDA formed/h/mg protein), SOD (units/mg protein) and CAT (μM of H_2O_2 decomposed/min/mg protein) activities in male mouse. Vertical lines indicate standard errors of the means. *, $P < 0.01$ compared to the respective control values. #, $P < 0.01$ compared to the values of cadmium (Cd) treated animals.

following the cadmium treatment. Both cadmium and ashwagandha-treated mice showed decrease in LPO in both the tissue homogenates when compared with that of mice treated with cadmium only. The results clearly indicate that ashwagandha is capable of reducing the toxicity caused by cadmium. Further, we found a significant decrease in SOD and CAT activities after cadmium administration and nearly normal values were obtained when ashwagandha was administered along with cadmium.

As in other studies, in the present investigation also cadmium increased tissue LPO and decreased the activities of antioxidant enzymes such as SOD and CAT which are known as endogenous antioxidative enzymes¹⁹. However, when ashwagandha extract was administered in the metal intoxicated mice, LPO was decreased and nearly normal values of antioxidative enzymes (SOD and CAT) were observed, indicating the ameliorating effect of this plant extract in metal toxicity.

Some antioxidants such as vitamin E, ascorbic acid and GSH decrease free radical generation and increase SOD and CAT activities. These have been found to protect the metal-induced oxidative damage²⁰. However, no plant product has been reported earlier to regulate the cadmium-induced toxic effects. The present findings clearly indicate the protective role of ashwa-

gandha extract on cadmium toxicity in mice.

It is thus suggested that ashwagandha extract may prove to be useful in the regulation of metal-induced clinical toxicity.

1. Kirtikar, K. R. and Basu, B. D., *Indian Medicinal Plants* (eds Blatter, E., Caius, J. F. and Bhaskar, K. S.), Periodical Book Agency, Delhi, 1991, vol. 3, p. 1774.
2. Malhotra, C. L., Das, P. K. and Dhalla, N. S., *Indian Medicinal Plants* (eds Blatter, E., Caius, J. F. and Bhaskar, K. S.), Periodical Book Agency, Delhi, 1960, vol. 4, pp. 49-64.
3. Malhotra, C. L., Das, P. K. and Dhalla, N. S., *Indian J. Physiol. Pharmacol.*, 1960, 4, 35-38.
4. Singh, N., Singh, S. P., Singh, D. R., Gupta, M. L., Kohli, R. P. and Bhargava, K. P., *Int. J. Crude Drug Res.*, 1986, 24, 90-100.
5. Singh, R. H. and Tripathy, R. K., *J. Res. Ind. Med.*, 1982, 1, 23-28.
6. Dhawan, B. N., *Curr. Sci.*, 1995, 68, 202-204.
7. Uma Devi, P., *Indian J. Exp. Biol.*, 1996, 34, 927-932.
8. Friberg, L., *ANA Arch. Ind. Health*, 1959, 20, 401-407.
9. Funakoshi, T., Ohta, O., Shimada, H. and Kojima, S., *Toxicol. Lett.*, 1995, 78, 183-188.
10. Srivastava, R. C., Gupta, S., Ahmad, N., Hasan, S. K., Farookh, A. and Husain, M. M., *J. Toxicol. Environ. Health*, 1996, 47, 173-182.
11. Rana, S. V. S., Singh, R. and Verma, S., *Indian J. Exp. Biol.*, 1996, 34, 177-179.
12. Yoshida, K., Sugihira, N., Suzuki, M., Sakurada, T., Saito, S., Yoshinaga, K. and Saito, H., *Environ. Res.*, 1987, 42, 400-405.
13. Gupta, P. and Kar, A., *Bull. Environ. Contam. Toxicol.*, 1997, 58, 422-428.
14. Ohkawa, H., Ohishi, N. and Yagi, K., *Anal. Biochem.*, 1979, 95, 351-358.
15. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J., *J. Biol. Chem.*, 1951, 193, 265-275.
16. Marklund, S. and Marklund, G., *Eur. J. Biochem.*, 1974, 47, 469-474.
17. Aebi, H. E., in *Methods in Enzymatic Analysis* (ed. Bergmeyer, H. U.), Academic Press, New York, 1983, vol. 3, pp. 276-286.
18. Dixon, W. J. and Massey, F. J., in *Introduction to Statistical Analysis*, McGraw-Hill, New York, 1983, pp. 121-218.
19. Shukla, G. S., Hussain, T. and Chandra, S. B., *Pharmacol. Toxicol.*, 1987, 60, 355-359.
20. Tappel, A. L., *Am. J. Clin. Nutr.*, 1970, 23, 1137-1139.

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Lambornella (a ciliate parasite) for biological control of *Anopheles stephensi*, the urban malaria vector

Lambornella is a hymenostome endoparasitic ciliate, two species of which namely, *L. clarki* and *L. stegomyiae*, are known to cause ciliotosis in mosquito larvae^{1,2}. We reported the natural infection of immatures of *Anopheles barbirostris*, *Anopheles hyrcanus* gp. and *Anopheles philippinensis* s.l. by ciliate of genus *Lambornella* for the first time in Assam from a forest fringe village of district Dibrugarh³. We isolated the endoparasitic *Lambornella* from the naturally occurring infected larvae and have been culturing it in our laboratory successfully on biphasic medium of 2.8% nutrient agar with hay infusion. In

our entomological laboratory the cyclical mosquito colony of the urban malaria vector, *Anopheles stephensi* (Delhi strain) is also being maintained and we report here the occurrence of epizootic of ciliate parasite belonging to *Lambornella* genus in the colony of *Anopheles stephensi* due to the accidental infection which wiped out the mosquito colony completely.

In the month of September 1996, we initially noticed the gradual mortality of IV instar larvae of *Anopheles stephensi* affecting the pupal output. The mosquito colony was being maintained in enamel pans at $27 \pm 2^\circ\text{C}$ temperature

and $70 \pm 10\%$ relative humidity and immatures were fed on a diet of dog biscuit + yeast (60:40). Change in larval food did not stop the mortality of immatures. Meanwhile the larval mortality was also noticed in II and III instar larvae. The growth of immatures was slowed down and the larval period between two instars was lengthened to 7-8 days as compared to the normal duration of 1-2 days. The larvae became sluggish by the time of attaining the III instar stage and would not come frequently to the water surface for respiration though on tapping the pan they wriggled at the bottom. It led to the suspicion of some