

flowers¹¹, based on style lengths, for the production of seeds and wasps does not seem to hold true in monoecious figs¹⁶.

Nevertheless, it is important to note that variation in the style lengths of a species was three to four times more than that in the ovipositor length of their respective pollinator wasps (Table 1). Such variation for style lengths compared to ovipositor lengths has also been reported earlier^{6,7}. Though the reason for this difference is not immediately clear, it is not unlikely that selection has favoured greater variance in style lengths as a plant strategy in evolutionary conflict between the fig and the pollinator, on the allocation of flowers to wasp and seed production.

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ACKNOWLEDGEMENT. We sincerely thank Dr U. C. Abdurahman and Dr D. R. Priyadarshan, University of Calicut, for identifying the wasp species. PK was supported by a JRF from ICAR, New Delhi. KNG and RUS were partially supported by a DST grant, Govt of India and a McArthur grant (to K. S. Bawa, Boston)

Received 31 December 1994, revised accepted 23 March 1995

Uptake and tissue distribution of cadmium in albino rat after oral exposure to cadmium-contaminated edible mushroom and its effect on blood

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Pleurotus sajor-caju showed a fair amount of Cd²⁺ uptake from the metal-contaminated substrate. To study the uptake capacity, distribution and degree of accumulation of Cd²⁺ in different internal organs and blood, fungal-tissue-incorporated Cd²⁺ was administered orally to albino rats for a period of six weeks. Kidney and spleen exhibited maximum (5.40-5.50 µg g⁻¹ dry wt) uptake of Cd²⁺. In all cases depletion of Zn²⁺ was noted with increase in Cd²⁺ level. Cadmium caused reduction in body weight and increase in relative weight of kidney and spleen. Haematological changes included a sharp decline in the percentage of packed cell volume and in haemoglobin, and significant alteration in differential count. Metal uptake and toxicity were always higher when the standard diet was supplemented with inorganic Cd²⁺ instead of tissue-incorporated Cd²⁺.

BIOSPHERE is being increasingly contaminated by indiscriminate discharge of toxic heavy metals from various sources, the long effects of this practice will be hazardous to all living organisms. The use of metal-containing sprays, pesticides and fertilizers may also increase contaminants in the soil¹. It has been reported^{2,3} that the soil might get polluted with a variety of metals like As, Cd, Cr, Cu, Fe, Hg, Mn, Mo, Ni, Pb, Se, V and Zn, which are mostly coming from industrial sources; naturally, therefore, their concentration in the soil is higher in the vicinity of an industrial area. Sometimes, mercury emitted from a source into the atmosphere is absorbed by leaves and, subsequently, moves to the humus through fallen leaves. Mushrooms usually grow on soil and other natural substrates which are sometimes contaminated with various heavy-metal pollutants. The uptake of these heavy metals by different edible mushrooms from various substrates has been reported earlier⁴⁻⁶. But no information is available so far regarding the consumption of tissue-(mushroom)-incorporated heavy metals by mammals and their distribution and accumulation in different internal organs and blood. The present communication deals with (1) the uptake of Cd²⁺ by *Pleurotus sajor-caju*, an edible fungus, (2) the distribution of Cd²⁺ in different internal organs and blood after oral exposure in albino rats, (3) extent of Zn²⁺ depletion in soft tissues due to the presence of Cd²⁺ and (4) the changes in haematological characteristics of mammalian blood.

The standard diet of rats purchased from a local market was supplemented with dried sporocarp powder of *P. sajor-caju* in 1:1 proportion and the Cd²⁺ content of both the standard and the Cd²⁺-supplemented diets were

Table 1. Cd²⁺ content of diets supplied to albino rats, amount of diet consumed and its effect on body weight (all values \pm S.E.)

Group of rats	Diet fed	Cadmium content of the diet fed ($\mu\text{g g}^{-1}$ air dry wt)*	Total diet (g) (air dry wt) consumed (during 6 weeks)	Body weight [†] (g)	
				Initial wt ^a (0 day)	Final wt ^b (42 day)
I	Standard diet (S D)	2.50 \pm 0.00	252.00	46.00 \pm 2.45	130.50 \pm 7.49
II	S D. + untreated sporocarp (1:1)	1.25 \pm 0.00	252.00	46.50 \pm 1.94	124.50 \pm 2.50
III	S D. + Cd ²⁺ -contaminated sporocarp (1:1)	17.92 \pm 0.83	247.25	46.50 \pm 0.65	109.75 \pm 3.12
IV	S D. + inorganic [‡] Cd ²⁺ (1:1)	18.08 \pm 0.17	243.50	49.50 \pm 1.32	108.25 \pm 1.75

^a30-day old rat, ^b72-day old rat

*3 replicates/treatment

[†]4 replicates/treatment[‡]Inorganic Cd²⁺ \equiv that present in Cd²⁺-contaminated sporocarp.**Table 2.** Relative weight of organs in rats fed with Cd²⁺-contaminated sporocarps of *P. sajor-caju* (4 replicates/treatment)

Organ	*Relative organ wt (g kg ⁻¹ body wt)				C.D. value	
	Standard diet (S D)	S.D. + untreated sporocarp (1:1)	S D. + Cd ²⁺ -contaminated sporocarp (1:1)	S D. + inorganic Cd ²⁺ (1:1)	5%	1%
	Liver	43.38 \pm 0.75	45.99 \pm 2.59	40.84 \pm 2.92	39.64 \pm 1.46	N.S.
Kidney	6.67 \pm 0.34	7.10 \pm 0.32	8.62 \pm 0.50	8.89 \pm 0.17	1.26	1.77
Spleen	2.36 \pm 0.09	2.41 \pm 0.04	3.75 \pm 0.29	3.75 \pm 0.39	1.59	2.23
Pancreas	1.64 \pm 0.17	1.97 \pm 0.11	1.73 \pm 0.07	1.99 \pm 0.12	0.73	1.03
Adrenal	0.64 \pm 0.02	0.72 \pm 0.02	0.87 \pm 0.05	1.03 \pm 0.11	N.S.	N.S.

$$* \text{Relative organ wt} = \frac{\text{Organ wt (g)}}{\text{Body wt (g)}} \times 1000$$

N.S. Not significant.

estimated following the method of Raschnik⁷. Cadmium detection was made by atomic absorption spectrophotometer (AAS) (Perkin Elmer 2380) equipped with deuterium background corrector.

The methods of spawn production, cultivation of *P. sajor-caju* and application of Cd²⁺ on mushroom described by Purkayastha *et al.*⁶ were followed for the purpose.

The standard diet of rats was obtained from Lipton India Ltd., Bangalore. One-month-old albino rats (Swiss strain) weighing 45–50 g were obtained from authentic breeders and divided into 4 groups consisting of 4 rats in each. Each group of rats was fed with standard diet (S.D.)/S.D. + uncontaminated sporocarp/S.D. + Cd²⁺-contaminated sporocarp/S.D. + inorganic Cd²⁺ for 6 weeks. At the end of the 6-week experimental period, the animals were autopsied under mild chloroform anaesthesia and were killed by exsanguination from the abdominal aorta. The heparinized blood samples were collected and used for determination of the percentage of haemoglobin, the packed cell volume, and also for

total count and differential count, following standard procedure⁸. Immediately after evisceration, the liver, kidney, spleen and adrenal were weighed, the organ-body weight ratio was calculated and the Cd²⁺ content was determined⁹.

Groups I and II rats were fed with S.D. and S.D. + uncontaminated sporocarp of *P. sajor-caju* (1:1), respectively. These diets had a very low content of Cd²⁺ (1.25–2.50 $\mu\text{g g}^{-1}$ dry wt). Groups III and IV, fed with S.D. + tissue-incorporated Cd²⁺ and inorganic Cd²⁺, respectively, contained greater amount of Cd²⁺ (18 $\mu\text{g g}^{-1}$ dry wt). Usually, the total diet supplied to each rat during the 6-week experimental period was 252 g. A reduction of 24.9% in body wt was noted for rats fed with Cd²⁺ (Table 1). The relative weights of kidney and spleen, however, increased by 27% and 56%, respectively, in the Cd²⁺-fed groups; the relative weights of liver, on the other hand, decreased by 10% (Table 2).

The deposition of Cd²⁺ in liver and spleen was more in rats supplied with inorganic Cd²⁺ than in those with tissue-incorporated Cd²⁺. The internal organs and whole

Table 3. Comparison of haematological characteristics (\pm S.E) of albino rats before and after feeding Cd^{2+} -contaminated sporocarps of *P. sajor-caju*

Group of rats	Diets supplied	Haemoglobin content of blood* (g/100 g)	Clotting time* (sec)	Total count* ($\times 1000\text{ ml}^{-1}$)	Differential count (%)						Packed cell volume (%)
					Neutrophil	Eosinophil	Basophil	Lymphocyte	Monocyte		
I	Standard diet (S.D)	15.69 \pm 0.10	19.00 \pm 0.91	14.15 \pm 0.06	21.25 \pm 1.38	1.70 \pm 0.30	0.50 \pm 0.29	74.75 \pm 1.93	1.80 \pm 0.27	49.15 \pm 0.63	
II	S.D + untreated sporocarp	15.39 \pm 0.22	15.00 \pm 0.41	13.65 \pm 0.31	23.50 \pm 1.04	1.35 \pm 0.54	0.45 \pm 0.18	73.88 \pm 0.55	1.33 \pm 0.45	52.75 \pm 1.03	
III	S.D + Cd^{2+} -contaminated sporocarp	14.62 \pm 0.37	20.00 \pm 0.41	14.28 \pm 0.30	28.75 \pm 1.79	0.95 \pm 0.41	0.23 \pm 0.19	69.25 \pm 1.79	0.83 \pm 0.28	48.75 \pm 1.31	
IV	S.D + inorganic Cd^{2+}	13.23 \pm 0.24	24.50 \pm 1.08	14.45 \pm 0.25	35.00 \pm 4.25	1.58 \pm 0.21	0.23 \pm 0.13	60.88 \pm 4.11	1.58 \pm 0.23	37.75 \pm 2.02	
CD values		5%	0.89	2.69	NS	NS	NS	NS	NS	NS	
		1.25	3.74	NS	NS	NS	NS	NS	NS	6.71	

*Average of 4 replicates/treatment

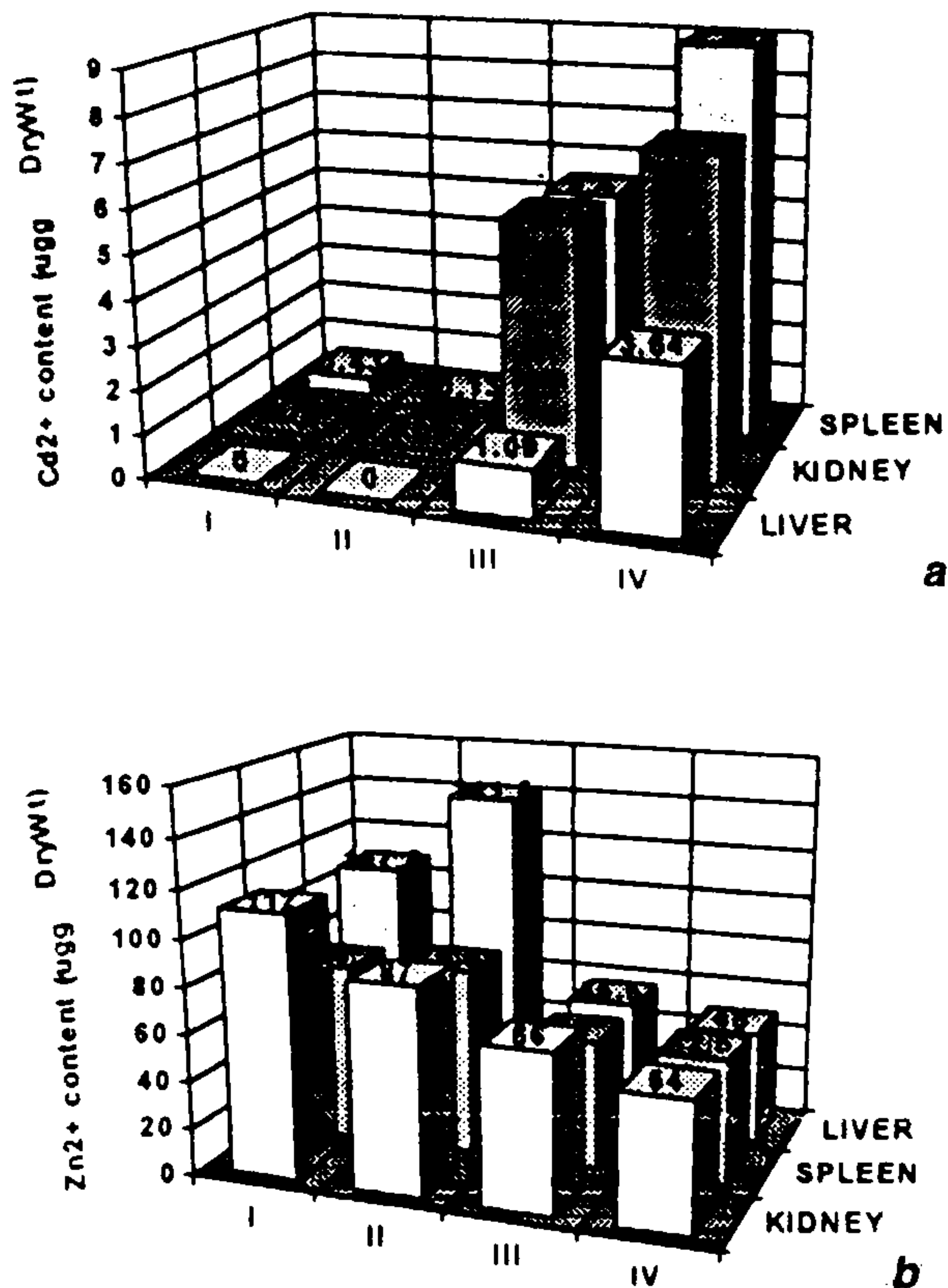


Figure 1. a. Cadmium content of internal organs of albino rats after feeding the Cd^{2+} -contaminated mushroom diet (*P. sajor-caju*) b. Zinc content of internal organs after feeding the same 1. Standard diet (S.D), II S.D + untreated sporocarp (I I), III S.D + Cd^{2+} -contaminated sporocarp (I I), IV S.D + inorganic Cd^{2+} (I I)

blood showed moderate (31.50%) to high (52.73%) depletion of zinc due to increasing Cd^{2+} concentration (Figure 1 a, b).

The results in Table 3 indicate the haematological irregularities due to the consumption of Cd^{2+} -contaminated diets. The signs of anaemia (fall in PCV%, Hb%) were apparent. Besides, there was a gradual increase in the percentage of neutrophil and a proportionate reduction in the percentage of lymphocyte. There was no significant change in clotting time and total count.

Acute Cd^{2+} toxicity caused by food consumption is rare, but chronic exposure to high Cd^{2+} levels could significantly increase the accumulation of Cd^{2+} in certain internal organs¹⁰. The loss in body weight appeared to be the first symptom of Cd^{2+} toxicity, this has been reported earlier also¹¹. In 1990, Groten *et al.*¹² demonstrated that inorganic Cd^{2+} and pig's-liver-incorporated Cd^{2+} produced similar toxicity in rats, which included reduction in relative weight of liver. In the present study, development of hepatocytic lesions was also no-

ted. Previous workers^{13,14} have pointed out that a correlation exists between reduction in Cd²⁺ toxicity and increased level of Zn²⁺ in the tissues. The present results reveal that increased level of Cd²⁺ caused toxicity and depletion of zinc in the tissues. Apart from this, Cifone *et al.*¹⁵ noted reduction of large granular lymphocytes in the peripheral blood of Cd²⁺-treated rats; this was also confirmed. The results reveal that edible mushrooms can absorb fair amount of Cd²⁺ from the substrates, if present, and the consumption of Cd²⁺-contaminated mushroom by mammals may cause differential accumulation of the same in different internal organs like liver, renal cortex and blood, causing health hazards. But the degree of accumulation depends on the duration of exposure, the nature of metal species and their concentration, and the nature of animal tissues.

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ACKNOWLEDGEMENTS We thank the University Grants Commission, New Delhi, for providing financial assistance during the execution of this work

Received 10 November 1994, accepted 7 March 1995

Effect of vincristine on Leydig cell and accessory reproductive organs

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The effect of vincristine (VCR), currently in use as a mitotic spindle poison in combination chemotherapeutic regimens for cancer, on the Leydig cell and the accessory reproductive organs has been tested in the light of the reports that it affects spermatogenesis. VCR was administered to Wistar strain male albino rats at a daily dose of 20 µg for 15 days. Testis, caput and cauda epididymides, seminal vesicle and ventral prostate were prepared for light microscopic observation; slices of testis were also subjected to electron microscopic analysis; the cholesterol content of the testis and the fructose content of the seminal vesicle were also determined. The results show that seminal vesicle and ventral prostate were regressed. Lumen of the caput epididymis lacked sperm but contained giant cells; in the cauda, giant cells appeared disintegrating. Secretory acini/follicles of the seminal vesicle/ventral prostate exhibited decreased secretory activity. Fructose content of the seminal vesicle also decreased. Cytoplasm of Leydig cell of treated rats appeared highly vacuolated and the nucleus, chromatin-depleted. Therefore, the regression and other derangements in the accessory reproductive organs appear to be manifestation of the toxic

effect of the drug on Leydig cell. Thus, the present paper reports for the first time VCR toxicity to Leydig cell.

VINCRISTINE (VCR) is an indole-indolin dimeric alkaloid obtained from the West Indian periwinkle *Vinca rosea* Linn.¹; it is also synthesized from another *Vinca* alkaloid, vinblastine, through Polonovski reaction². This substance was introduced as a chemotherapeutic in cancer treatment by Johnson *et al.*¹; subsequently, it has come to stay as one of the drugs in combination chemotherapy for several kinds of cancers³. Various toxic effects/side-effects like nausea/vomiting, alopecia, diarrhoea, anaemia, hepatocellular damage, pulmonary fibrosis, myocardial infarction, hyponatremia, peripheral neuropathy, etc., have been reported for VCR⁴⁻⁶. However, studies on the male reproductive toxicity of this drug have been attempted only sporadically and all the earlier studies conducted are related only to the spermatogenic compartment of the testis; the effects reportedly include inhibition of thymidine, uridine and L-leucine incorporation in all testicular cell types, accompanied with decrease in fertility, without affecting the spermatozoa⁷, increase in the amount of abnormal sperm with no stem cell killing⁸, dose-dependent reduction in the number of surviving seminiferous tubules with topographic variation⁹, arrest of mitotic and meiotic division at metaphase followed by cell death consequent to impact on Sertoli cell¹⁰ and generation of giant spermatogenic cells^{11,12}. Thus, the Leydig cells as well as the accessory reproductive organs, which are androgen-dependent, have been practically ignored in the