Table 1 *Phosphate solubilization by pure and mixed cultures in Pikovskaya's medium*

	Period of incubation (days)							
	7		14		21		28	
	pH of medium	Soluble P (mg/100 ml)	pH of medium	Soluble P (mg/100 ml)	pH of medium	Soluble P (mg/100 ml)	pH of medium	Soluble P (mg/100 ml)
A. chroococcum	5.82	5.7	5.96	8.0	6.00	8.7	5.86	9 6
P. striata	4 90	25.0	5.20	17.2	5.56	12.1	5.91	10.8
A. chroococcum + *P. striata*	4.81	28.9 (15.6)*	5.08	19.1 (11.0)	5.63	13.0 (7.4)	5.80	11.6 (7.4)

*Per cent increase over *P. striata* alone.

Table 2 *Nitrogen fixation by pure and mixed cultures in Jensen's medium*

	mg N fixed/g sucrose oxidized			
	Period of incubation (days)			
	15	30	45	60
A. chroococcum	20.0	19.5	16.1	12.5
P. striata	1.3	6.2	4.3	4.5
A. chroococcum + *P. striata*	17.8	17.3	15.3	11.1

Azotobacter growth was stimulated owing to the presence of available phosphorus in the culture medium. The results also indicate an inverse correlation between phosphate solubilization and pH of the medium. With the increase in pH with time of incubation, there was a decrease in phosphate solubilization[2].

Estimates of nitrogen fixation by pure and mixed cultures (table 2) revealed that *A. chroococcum* alone, fixed a fairly high amount of nitrogen in Jensen's liquid medium, ranging from 20.0 mg/g of sucrose oxidized at 15 days to 12.5 mg at 60 days. Surprisingly, association of *A. chroococcum* with *P. striata* did not improve the nitrogen-fixing capacity of *Azotobacter* and the quantity of nitrogen fixed was less than that by *A. chroococcum* alone. This is in conformity with an earlier finding[3] and could probably be due to assimilation of the fixed nitrogen by *Pseudomonas.*

Thus, synergistic interaction of *P. striata* and *A. chroococcum* in increasing phosphate solubilization may prove beneficial for developing a mixed inoculant for increasing crop productivity.

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LEVELS OF SERUM ANTIOXIDANTS IN DOXORUBICIN-TREATED RATS—INFLUENCE OF VITAMINS E AND C

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DOXORUBICIN (also known as adriamycin) is a broad-spectrum chemotherapeutic agent effective against human malignancies such as leukaemias, lymphomas and many solid tumours[1]. Formation of free radicals as well as accumulation of lipid peroxides have been well documented in heart[2] and serum[3] of patients and experimental animals under doxorubicin therapy. The increased peroxidation of polyunsaturated fatty acids is recognized as one of the possible biochemical mechanisms of genesis of membrane injury in the myocardium[4]. Generally, iron in its free form is believed to accelerate lipid peroxidation induced by free radicals[5]. A major part of extracellular antioxidant defence is to keep this ionic iron largely sequestered in proteins[6]. Albumin, haemopexin and haptoglobin have been shown to inhibit various radical reactions probably by sequestering metals[6].

Since the deleterious effects produced by free radicals depend upon the balance between oxidant and antioxidant capacity of the system, we investigated the levels of the extracellular antioxidants uric acid, ceruloplasmin and albumin, and total iron-binding capacity. Serum iron and lipid peroxides

were also measured and compared with rats co-administered vitamins E and C.

Doxorubicin was purchased from Sigma Chemicals, USA. Male albino rats, derived from Wistar strain, weighing 90–100 g, were selected and divided into 3 groups. The animals were allowed free access to food and water. Group I served as control. Group II and III animals were treated with doxorubicin 2 mg/kg body weight twice weekly for 4 weeks intravenously[7]. Group III animals also received vitamin E (40 mg/rat/day) and vitamin C (100 mg/rat/day). The vitamin therapy was carried out for 2 months. After the experimental period the animals were killed by cervical dislocation and the blood was collected and serum separated. Whole blood (0.5 ml) was taken for glutathione[8] estimation. Albumin[9], ceruloplasmin[10], uric acid[11], iron[12] and total iron-binding capacity[13] in serum were measured. Serum lipid peroxides were measured by the method of Yagi[14]. Vitamin E was determined by the method of Desai[15] and vitamin C by the method of Kyaw[16].

The levels of serum lipid peroxides, iron and total iron-binding capacity are presented in table 1. A significant increase in lipid peroxides and iron content is evident in group II animals, which could be attributed to the low iron-binding capacity of the serum. The increased circulating iron may have been responsible for induction of free radical formation. Vitamin-treated animals show decreased lipid peroxide, which shows the efficacy of these vitamins as antioxidants. Iron-binding capacity is also increased under vitamin therapy.

Table 2 shows the levels of ceruloplasmin, albumin and uric acid in control and experimental groups. The levels of these antioxidants decreased considerably in group II animals. Ceruloplasmin oxidizes Fe(II) to Fe(III), which swiftly binds to transferrin[17] and is ineffective in inducing free radical reactions. Uric acid and albumin[18] have also been shown to be good chelators of metal ions which promote lipid peroxidation. We have already reported low level of serum protein[19] in doxorubicin-treated rats.

Table 1 *Levels of serum lipid peroxides, iron and total iron-binding capacity in control and doxorubicin-treated albino rats*

Group	Serum lipid peroxides (nmol MDA/ml)	Serum iron (μg/100 ml)	Total iron-binding capacity (%)
Control	2.1 ± 0.05	40.5 ± 2.1	71 ± 1.2
Doxorubicin	4.0 ± 0.06**	63.2 ± 4.1*	49 ± 1.1*
Doxorubicin + vitamins E and C	1.83 ± 0.02	45.3 ± 2.3	65 ± 2.3

Values are mean ± SD ($n = 6$ in each group).
*$P < 0.01$, **$P < 0.001$.

Table 2 *Levels of serum ceruloplasmin, uric acid and albumin in control and doxorubicin-treated albino rats*

Group	Ceruloplasmin (units/ml)	Albumin (g/dl)	uric acid (mg/dl)
Control	0.931 ± 0.056	3.56 ± 0.10	3.8 ± 0.15
Doxorubicin	0.506 ± 0.01*	2.05 ± 0.15*	2.5 ± 0.13*
Doxorubicin + vitamins E and C	1.405 ± 0.03	4.21 ± 0.13	3.6 ± 0.19

Values are mean ± SD ($n = 6$ in each group).
*$P < 0.01$.

Table 3 *Levels of blood glutathione and serum vitamins E and C in control and doxorubicin-treated albino rats*

Group	Glutathione (mg/100 ml)	Vit. E (mg/100 ml)	Vit. C (mg/100 ml)
Control	60.1 ± 1.5	1.050 ± 0.07	1.82 ± 0.07
Doxorubicin	42.3 ± 2.1**	0.750 ± 0.01**	1.69 ± 0.03*
Doxorubicin + vitamins E and C	63.5 ± 2.9	1.490 ± 0.06	2.00 ± 0.04

Values are mean ± SD ($n = 6$ in each group).
*$P < 0.05$, **$P < 0.01$.

The levels of glutathione and vitamins E, C are given in table 3. A significant decrease in glutathione level is noted in doxorubicin-treated animals. Consumption of glutathione[20] during free radical-induced reactions has been well demonstrated. The activation of lipid peroxidation may have resulted in increased requirement of tissues for vitamin E and thereby decreased serum vitamin E content. Vitamin-treated animals show high vitamin E content. The level of vitamin C is not greatly elevated, probably due to continuous utilization for the regeneration of vitamin E[21]. The level of glutathione, which is an effective free-radical scavenger, is maintained in vitamin-treated animals.

From these results it can be concluded that doxorubicin or its metabolites tilt the balance towards auto-oxidation by reducing antioxidant levels, and vitamins E and C reverse this trend by accelerating the effects of metal chelators and also by their own antioxidant capacity.

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ANTIFERTILITY EFFECT OF *VINCA ROSEA* (LINN.) LEAF EXTRACT ON MALE ALBINO MICE—A SPERM PARAMETRIC STUDY

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VINCA ROSEA (Linn.) (*Catharanthus roseus*), of the family Apocynaeceae, with proven efficacy as anticarcinogenic[1], has also been shown to be antispermatogenic and antiandrogenic in rats[2,3]. A preliminary study[3] has shown that aqueous extract of leaves of this plant affects motility of rat sperm. The present paper reports the effect of aqueous extract of leaves of this plant on various sperm parameters of Swiss albino mice.

Male Swiss albino mice (8 weeks old, 30–38 g body weight), fed on standard mouse pellet diet (Hindustan Lever Ltd, Bombay) and water, were used. Fresh leaves of *V. rosea* were collected locally. Aqueous leaf extract was obtained according to Chinoy and Geetha Ranga[3]. Mice ($n=10$) in the experimental group received 10 mg equivalent of the dry leaf in 0.2 ml of water daily intraperitoneally for 15 days. Control mice ($n=10$) received physiological saline. The animals were sacrificed 24 h after the last injection by cervical dislocation and the cauda epididymides were removed. Modified Kreb's Ringer bicarbonate solution containing crystalline bovine serum albumin[4] was used as the medium for *in vitro* study of sperm. Cauda epididymis was placed in 200 μl of the buffer taken in an embryo cup. The cauda was punctured and minced using fine needles to release and disperse the sperm. Tissue debris was discarded, leaving a clear preparation for microscopic observation. Hanging drop preparations were made to assess sperm motility, relative percentage of live and dead sperm and sperm count; smears were prepared to assess relative proportions of normal and abnormal sperm[5].

V. rosea leaf extract did not affect body weight of the mice. Sperm count of the treated mice decreased significantly to 67% of that of controls. The relative percentage of motile sperm decreased on treatment.

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