facultative apomicts, attempts are being made to detect and isolate stable apomicts in sorghum.

The progeny test made by Reddy *et al.* (Reddy, C. S., Schertz, K. F. and Bashaw, E. C., *Euphytica*, 1980, **29**, 223–226) relied on converting R473 into genetic male steriles. It is not clear how the study of progeny of male sterile mutants with normal R473 (as male) can give insight into the reproductive behaviour of R473. Full explanation of these findings has already been given in Murty *et al.*, 1982 (Murty, U. R., Rao, N. G. P., Kirti, P. B. and Bharathi, M., in *Sorghum in the Eighties*, ICRISAT, Hyderabad, 1982, 361–372).

Vybrids

The concept of vybrids (facultative apomicts) was only of an exploratory nature with the objective of using facultative apomixis for partial fixation of heterosis. It was never the objective of this work to develop vybrids to compete with commercial hybrids of sorghum.

Relevant recent work in cereal grain crops

There has been growing interest globally in the exploitation of apomixis in heterosis breeding of rice, maize, wheat, sorghum, pearl millet, etc. During 1992 itself three International Workshops on Apomixis were conducted, IWAR (International Workshop on Apomixis in Rice in China), ABCI (Apomixis Biology and Crop Improvement, at Atlanta, Georgia, USA) and APONET (First International Workshop on Potential use for Apomixis in Tropical Plant Breeding at Montpellier, France) which reflect the current interest. Our work on apomixis in sorghum was presented and discussed at the First APONET Workshop in France.

We realize that our own embryological and cytogenetical studies, reported in well-refereed journals periodically as they were observed, did alter the initial finding. In this context, it may not be out of place to conjecture that such alterations have been inherent in analyses aimed at enhanced understanding of this complex phenomenon.

As examples, Petrov and his colleagues introduced apomixis in maize which was reported to have been lost over generations. Savidan writes: 'Petrov's material is a great contribution to apomixis research. The fact that they lost apomixis in later generations is no dishonour. Petrov and his colleagues worked with the tools they had and based their information on the information available to them at that time' (Savidan, Y., *Apomixis Newsl.*, 1991, **3**, 26–27). Similarly the existence of apomixis and its exploitation in rice has been widely reported in China but confirmatory evidence has been lacking (Chen, J. S., *Apomixis Newsl.*, 1991, **3**, 8).

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RESEARCH COMMUNICATIONS

Influence of α-tocopherol on the antitumour potential and the toxicity of doxorubicin

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α-tocopherol reduces the lipid-peroxidation induced in doxorubicin therapy. It is aimed at here in this paper to furnish evidence for the protecting effect of α-tocopherol over doxorubicin-induced toxicity. Such protection is of value only if the antitumour potential of doxorubicin is not significantly reduced by α-tocopherol.

Doxorubicin is an antitumour antibiotic clinically active against a wide spectrum of experimental and human solid tumours[1]. The therapeutic usefulness of doxorubicin is seriously limited by its cardiotoxic and other side effects[2]. The chronic effects include insidious onset of cardiomyopathy which often leads to congestive heart failure[3].

Doxorubicin is known to generate superoxide radicals either enzymatically or non-enzymatically and to stimulate the formation of lipid peroxides[4]. Since lipid peroxidation induced by doxorubicin has been considered as the proposed machanism for the deleterious side effects, various antioxidants have been tried along with doxorubicin[5]. α-tocopherol has been shown to reduce the lipid-peroxidation induced in doxorubicin therapy[6].

Serum, heart and liver LDH levels and its isoenzymes are of clinical interest because they can be used as molecular markers of tissue damage. Since liver is the major organ involved in drug metabolism and heart the target organ for this drug, the LDH total activity and its isoenzymes are assessed in liver, heart and also in serum. The present study is aimed at furnishing

evidence for the protecting effect of α-tocopherol over doxorubicin-induced toxicity.

It is necessary to prove that such protection against doxorubicin-induced toxicity is of value only if the antitumour potential of doxorubicin is not significantly reduced by α-tocopherol. Hence, the influence of α-tocopherol on the antitumour effect of doxorubicin is also investigated using fibrosarcoma tumour model.

Doxorubicin and α-tocopherol were purchased from Sigma Chemical Company, USA. Male albino rats derived from Wistar strain, weighing 90–100 g were selected and divided into 4 groups. The animals were allowed free access to food and water. Group 1 served as control, group 2 rats received doxorubicin (2.5 mg/kg body wt., weekly once for 8 weeks, I. V.)[7], group 3 rats were given α-tocopherol orally (400 mg/kg body wt., daily for 2 months),[8] and group 4 animals received both doxorubicin and α-tocopherol.

After the experimental period, rats were killed by cervical decapitation and blood was collected. Liver and heart were excised and homogenized in tris-HCl buffer 0.1 M pH. 7.4. LDH activity was determined by the method of King[9]. Isoenzymes were separated by the method of Ornstein[10] and stained by the method of Dietz[11]. Protein was estimated by the method of Lowry *et al.*[12]

Fibrosarcoma was induced and maintained in rats by serial transplantation. After a week of transplantation the rats were divided into 4 groups and treated as in the toxicity study. The survival time was carefully noted and recorded.

The antitumor potency was assessed by *in vitro* ^{3}H-thymidine uptake test. At definite time intervals lipid peroxide contents were measured[13].

LDH activities in serum, liver and heart are given in Table 1. Doxorubicin treatment resulted in a significant increase in serum LDH activity. Serum LDH level is a diagnostic indicator of myocardial and liver functional disorders and the quantity of enzyme released is a measure of the number of necrotic cells[14]. Lipid peroxidation has been reported to be a cause for tissue necrosis in general. We have also observed liver and heart necrosis in doxorubicin treatment[15]. So this could have resulted in the leakage of LDH into the blood stream. In α-tocopherol co-administered rats there is no significant change when compared to control rats.

LDH is a —SH group containing enzyme[16] and this could have resulted in the inactivation of liver and heart LDH by the active free radicals formed during doxorubicin metabolism. -SH groups have been reported to be susceptible for free radical attack[17] and α-tocopherol has been proved successful in protecting the -SH groups of biomolecules from oxidative damage[18].

Doxorubicin treatment resulted in sharp increases in all the isoenzymes, but significantly in liver and heart enzymes. In group 4 the elevation is minimized (Figure 1).

Table 2 presents the survival time of tumour bearing rats treated with doxorubicin and α-tocopherol. Administration of doxorubicin effectively improves the survival time. Doxorubicin + α-tocopherol-treated rats show comparatively prolonged survival time.

Being the most potent drug in the field of cancer chemotherapy, the long term use of doxorubicin has

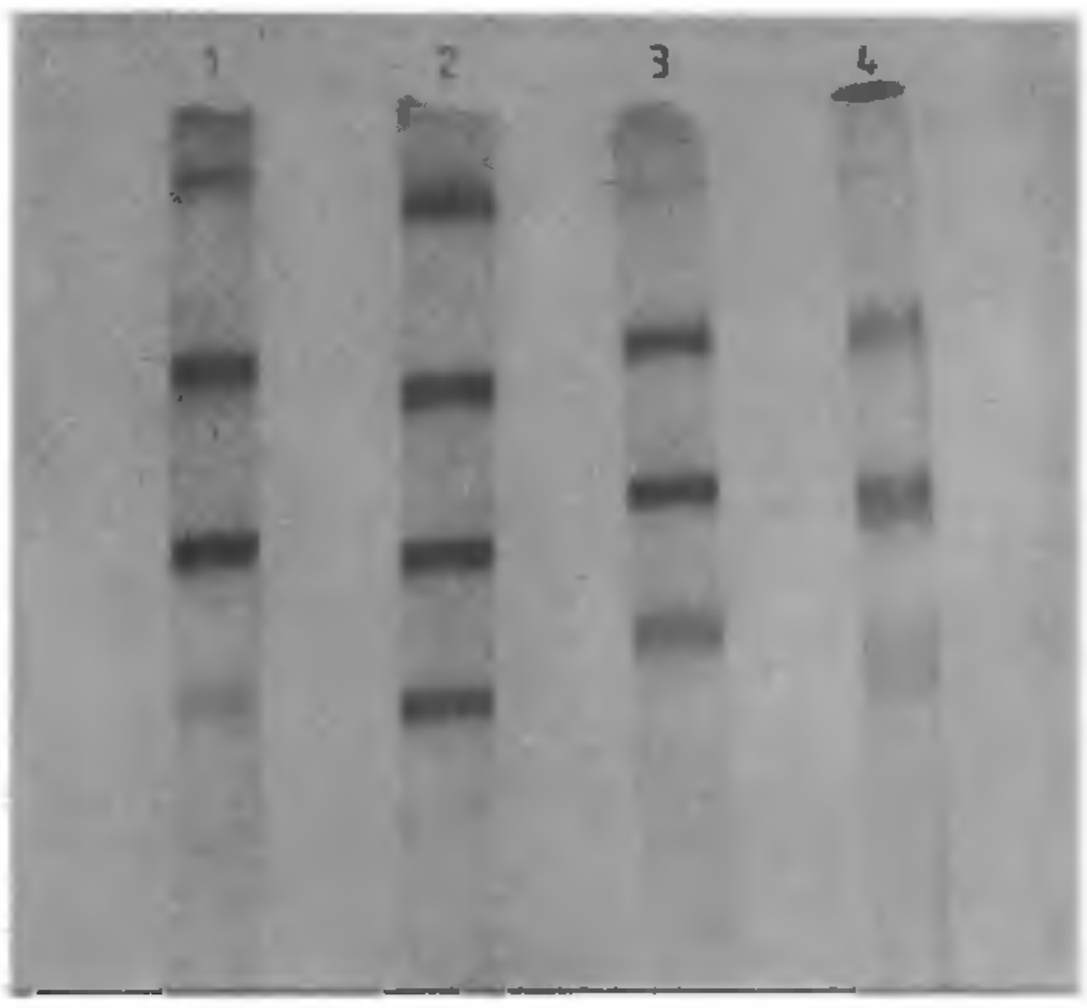

Figure 1. Serum LDH isoenzyme pattern. (1) Control; (2) Doxorubicin; (3) α-tocopherol; (4) Doxorubicin + α-tocopherol.

Table 1. Activities of serum, liver and heart LDH in control and experimental rats.

Group	Serum (IU/litre)	Liver (n moles pyruvate liberated/min/mg protein)	Heart (n moles pyruvate liberated/min/mg protein)
Control	82.6 + 4.3	1310 + 79	123.3 + 9.8
Doxorubicin	105.0 + 7.3*	910 + 63.5*	87.9 + 4.4*
α-tocophl.	79.3 + 5.6	1350 + 101^{NS}	129.5 + 10.2^{NS}
Doxorubicin + α-tocopherol	80.6 + 5.2**	1240 + 83.5^{NS}	109.6 + 5.6**

Values are mean ± SD (n = 6 in each group)
*p 0·001; **p 0·05; NS Not significant

Table 2. The mean survival time of control and experimental rats

Group	Tumour-bearing rats treated with	Survival time (days)
1	None	75 + 10 (n = 30)
2	Doxorubicin	163 + 23 (n = 28)
3	α-tocopherol	80 + 91 (n = 32)
4	Doxorubicin + α-tocopherol	200 + 25* (n = 30)

*p < 0·01 (Group 2 vs 4)

been limited by the onset of lipid peroxidation which results in cardiotoxicity. The prolonged survival time noted in group 4 animals might have been due to the amelioration of side effects associated with doxorubicin therapy.

^{3}H-thymidine uptake which is an index of DNA synthesis was decreased significantly at 500 μM doxorubicin concentration (Figure 2). α-tocopherol did not modulate this effect. There was a time and dose-dependent increase in lipid peroxide level in doxorubicin-treated fibrosarcoma cells. α-tocopherol reduced lipid peroxide formation (Figure 3).

In the presence of α-tocopherol doxorubicin-induced elevation of lipid peroxidation was inhibited to some extent but the magnitude of DXR-induced inhibition of DNA synthesis was not altered significantly. So it could be stated that the cytotoxic nature of doxorubicin is not influenced by its lipid peroxidizing ability.

From these results it is evident that the antitumour property of doxorubicin is not altered by α-tocopherol. Also the toxic effects of doxorubicin are minimized by α-tocopherol. So the combined therapy with doxorubicin and α-tocopherol might be more effective in cancer treatment.

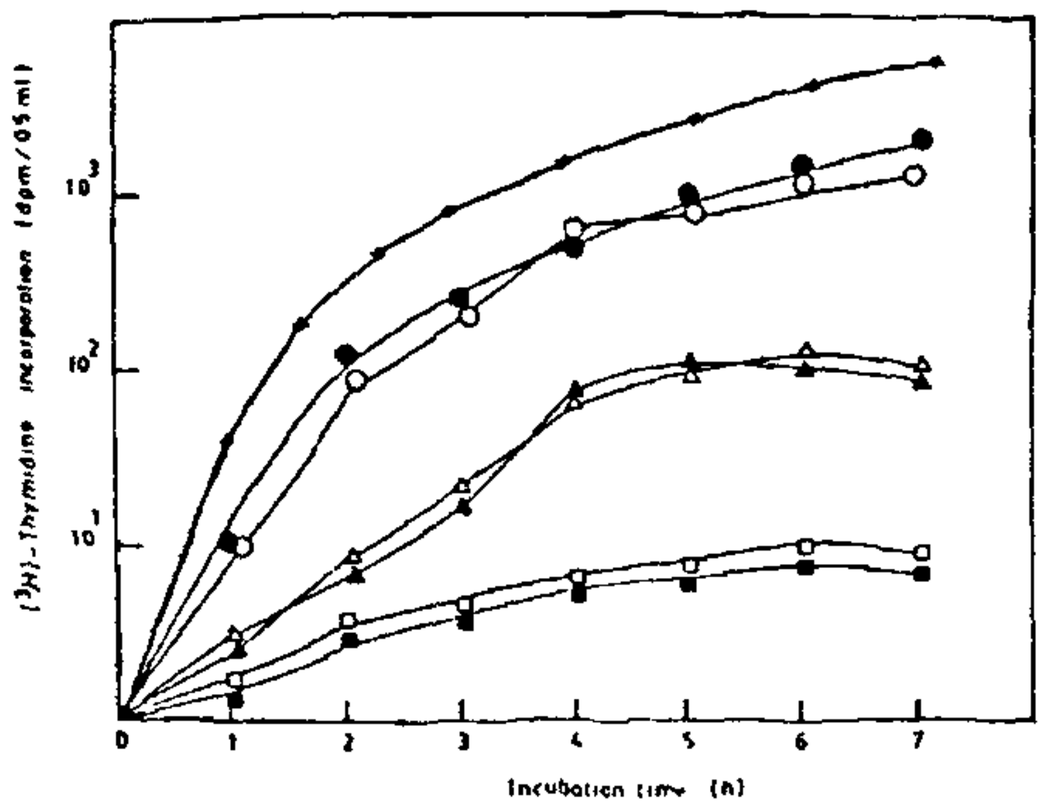

Figure 2. Effect of α-tocopherol on the doxorubicin-induced suppression in ^{3}H-thymidine incorporation into fibrosarcoma cells. ■, 500 μ mole doxorubicin; □, 500 μ mole doxorubicin + 100 mmole α-tocopherol; △, 200 μ mole doxorubicin; ▲, 200 μ mole doxorubicin + 100 mmole α-tocopherol; ○, 100 μ mole doxorubicin; ●, 100 μ mole doxorubicin + 100 mmole α-tocopherol; ★, control.

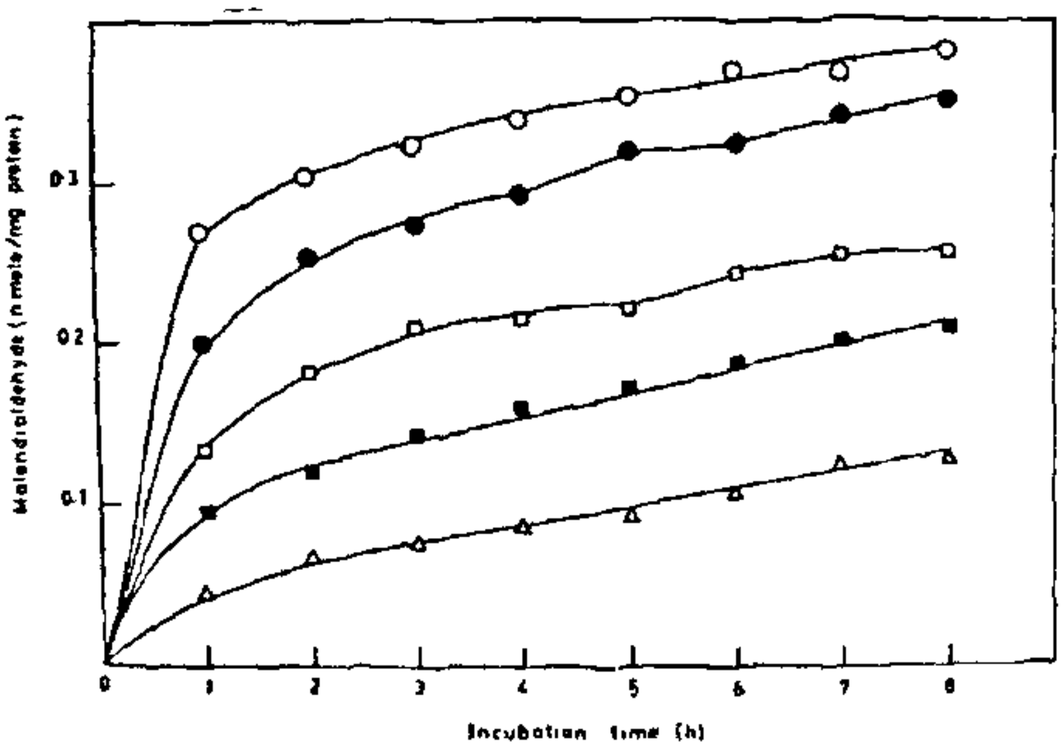

Figure 3. Effect of α-tocopherol on doxorubicin-induced lipid peroxidation in fibrosarcoma cells. △, Control; ○, 500 μ mole doxorubicin; ●, 500 μ mole doxorubicin + 100 mmole α-tocopherol; □, 200 μ mole doxorubicin; ■, 200 μ mole doxorubicin + 100 mmole α-tocopherol.

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