Lack of solid tumour protection by Ocimum extract and its flavonoids orientin and vicenin

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Ocimum leaf extract and its flavonoids orientin and vicenin protect normal tissues against radiation injury at nontoxic doses. For application in cancer patients, they should not protect tumours. Effect of extract (50 mg/kg) was studied on mouse fibrosarcoma treated with radiation (10 Gy), cisplatinum (5 mg/kg) and withaferin A (40 mg/kg) by lung colony assay. Effect of orientin and vicenin (50 µg/kg) was studied on fibrosarcoma and B16F1 melanoma treated with radiation (30 Gy) or cyclophosphamide (300 mg/kg) by tumour regression and mouse survival. Neither the extract nor flavonoids gave any protection to tumours, suggesting that they are suitable for normal tissue protection in cancer therapy.

RESEARCH on the radioprotective effect of natural products has indicated the potential of medicinal and dietary plants in the development of nontoxic radioprotectors for human application. Our earlier investigations on the Indian medicinal plant *Ocimum sanctum* (Krishna tulasi) have demonstrated that an aqueous extract of the dried leaves and two water-soluble flavonoids, orientin and vicenin, isolated from the extract, have good normal tissue protective effect on mouse tissues. When injected 30 min before whole body lethal irradiation, the extract and flavonoids increased the 30-day mouse survival, giving dose modification factors (DMF) of 1.28 for the extract[1] and 1.3 and 1.37 respectively, for orientin and vicenin[2]. The optimum dose for protection was 50 mg/kg body weight of the extract (< 1/100th its LD_{50} in mice) and 50 µg/kg body weight of either flavonoid (even 100 mg/kg did not produce any toxicity in mice). The optimum dose of the extract gave an equal protection to mouse chromosomes as 300 mg/kg of WR-2721, while a combination of the two synergistically increased chromosome protection, at the same time eliminating the delayed chromosome toxicity of WR-2721 (ref. 3). Both orientin and vicenin at 50 µg/kg body weight gave equal protection as 150 mg/kg body weight of WR-2721 to bone marrow chromosomes of mouse, whole body exposed to 2 Gy gamma radiation[4]. Both the extract and the flavonoids have been demonstrated to have good antioxidant activity *in vitro* and antilipid peroxidative activity *in vivo*[5–7]. These studies indicate the potential of Ocimum products for normal tissue protection in cancer patients. But to be eligible for use in cancer therapy, a protector should give protection only to normal tissues without interfering with the therapeutic effect on tumours. Therefore, a study was conducted to see if protection by Ocimum extract and the flavonoids is confined to normal tissues, using transplanted mouse tumours B16F1 melanoma and fibrosarcoma treated with gamma radiation or chemotherapeutic drugs.

Two strains of mice, Swiss albino and C57BL/6J, were used. The animals were random-bred and maintained under controlled conditions of light (light : dark :: 12 : 12), temperature (23 ± 2°C) and humidity (55 ± 5%) in an air-conditioned animal house in the Department of Radiobiology, Kasturba Medical College, Manipal. Six to eight-week-old animals were selected for the experiments. All animal studies were conducted according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals, Government of India.

B16F1 melanoma was obtained from the Cancer Research Institute, Mumbai and propagated by subcutaneous injection of 10^5 cells on the dorsal skin of C57BL mice. Fibrosarcoma was obtained from R. Kuttan, Amala Cancer Research Centre, Trichur and propagated in adult Swiss albino mice. Tumour growth was monitored by measuring three diameters ($D1$, $D2$, $D3$) of palpable tumours and calculating the tumour volume (V) as follows: $V = \eth/6\ D1.D2.D3$. Tumours of 100 ± 10 mm^3 were used for the experiments.

Local irradiation of tumours was done in a field of 4″ × 4″ (the rest of the body was shielded) and a dose rate of 1.6 Gy/min from a ^{60}Co teletherapy source (Siemens, Germany) at the Sirdi Sai Baba Cancer Hospital, Manipal.

Fresh leaves of the dark-leafed variety of *O. sanctum* were collected locally, shade-dried and powdered. The aqueous extract was prepared from the powder by refluxing in double distilled water (DDW) at 60–80°C. The extract was dried in Speed Vac vacuum concentrator (SC 110A, Savant, USA), as described earlier[1]. Orientin and vicenin were isolated from the extract by the method of Uma Devi *et al.*[2] (patented in India, patent no. 184300). The extract and the flavonoids were dissolved in DDW freshly before injection.

Withaferin A (WA) is a steroidal lactone found in the leaves and roots of the medicinal plant *Withania somnifera*. This compound has shown significant cytotoxic and radiosensitizing effects on transplanted mouse tumours *in vivo*[8–11] and mammalian cells *in vitro*[12]. WA was isolated from the alcoholic root extract of *W. somnifera* by the method of Subramanian and Sethi[13]. The dried powder was dissolved in a few drops of ethanol and then made up to the desired concentration using 5% carboxy methyl cellulose.

Cis-diammine-dichloro-platinum (CDDP) was purchased from Tamil Nadu Dadha Pharmaceuticals, Chennai. Cyclophosphamide was from Khandelwal Laboratories, Mumbai. Drug solutions were prepared by dissolving in physiological saline.

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The effect of Ocimum extract on the response of mouse fibrosarcoma to different anticancer treatments was studied. Swiss albino mice bearing fibrosarcoma were given the optimum protective dose[1] of 50 mg/kg body weight of Ocimum extract (OE) by intraperitoneal (i.p.) injection. Thirty minutes after the injection, five animals each were treated with 30 Gy of gamma radiation locally (RT) or injected i.p. with 5 mg/kg of CDDP or 40 mg/kg of WA. Groups of five animals each were treated with OE, CDDP, WA or RT alone. One group was maintained without any treatment (control). Tumour response was studied by the lung colony assay[14], as follows. One hour after treatment, the animals were sacrificed, tumours were dissected out and single-cell suspensions were made by mechanical dispersion[15]. The cells were counted on a hemocytometer and a known number of cells, mixed with an equal number of heavily irradiated (50 Gy of ^{60}Co gamma rays) tumour cells, was injected into the tail vein of recipient mice of the same strain. Three weeks after the tumour cell injection, the recipients were killed by cervical dislocation. The whole lung was dissected out and visible nodules (lung colonies) on each lobe were counted under a stereomicroscope (Leica, USA). Cell survival was calculated as the number of lung colonies per 10^4 tumour cells injected. Five recipients were used for each donor tumour. The data are expressed as the mean of five donors ± standard error of the mean.

Next, the effect of orientin and vicenin on the response of mouse tumours to radiation and cyclophosphamide was studied. Swiss albino mice bearing fibrosarcoma and C57BL mice bearing B16F1 melanoma were used in this study. Ten animals each with either tumour were treated as follows: Control: No treatment; Orientin (Ot) alone: A single i.p. injection of 50 µg/kg body weight of Ot; Vicenin (Vc) alone: A single i.p. injection of 50 µg/kg body weight of Vc; RT alone: A single dose of 30 Gy locally to the tumour; Cyclophosphamide (CP) alone: A single i.p. injection of 300 mg/kg of CP; Ot + RT: A single i.p. injection of 50 µg/kg body weight of Ot 30 min before 30 Gy local irradiation of tumour; Vc + RT: A single i.p. injection of 50 µg/kg body weight of Vc 30 min before 30 Gy local irradiation of tumour; Ot + CP: A single i.p. injection of 50 µg/kg body weight of Ot 30 min before 300 mg/kg CP i.p.; Vc + CP: A single i.p. injection of 50 µg/kg body weight of Vc 30 min before 300 mg/kg CP i.p.

Tumour volume was measured every alternate day. The treatment response was evaluated from the following parameters: Volume doubling time (VDT): the time in days required for the tumour to reach double the treatment volume; Growth delay (GD): the difference in time, in days, required for the control and treated tumours to reach five times the treatment volume; Complete response (CR): Complete regression of tumour at the primary site without regrowth within 120 days of observation; Partial response (PR): More than 50% regression in tumour size. Statistical analysis was done by Student's *t* test and one way ANOVA.

Effect of OE on the response of fibrosarcoma to RT, CDDP, and WA was studied. Injection of 10^4 tumour cells from normal control animals produced 19.7 lung colonies. OE alone, 50 mg/kg, did not produce any discernible change in the lung colony survival.

Radiation (30 Gy) produced a significant reduction in the lung colonies, to 13.4 colonies per 10^4 tumour cells inoculated. CDDP (5 mg/kg) treatment produced a similar effect, reducing the lung colonies to 13.6 per 10^4 tumour cells, while WA (40 mg/kg) was less effective in reducing the lung colonies, producing 17 colonies per 10^4 tumour cells. Combination of CDDP or WA with RT further reduced the colonies to 9.5 and 10.5 respectively, per 10^4 cells. OE treatment before RT, CDDP or WA or their combinations did not bring about any notable change in their tumour killing effect, as revealed by the lung colonies (Table 1).

The effect of Ot and Vc on the tumour response to RT and CP was studied. In the case of B16F1 melanoma (Table 2), none of the RT treatments produced any complete response. But 50–60% of the RT, Ot + RT and Vc + RT treated animals survived at 120 days, all with tumour. RT (30 Gy) resulted in VDT of 13 days (against 2 days in control) and a GD of about 19 days. Pretreatment with Ot or Vc produced a slight increase in these values (Ot + RT: VDT = 14 days, GD = 20.4 days; Vc + RT: VDT = 13.3 days, GD = 21.7 days), even though the difference was not significant.

CP alone produced no CR, while treatment with either Ot or Vc before CP resulted in 20% CR. None of the untreated animals survived beyond 25 days, while 40–50% of the CP, Ot + CP and Vc + CP treated animals survived up to 120 days, 20% of which in the Ot/Vc + CP groups without tumour (Table 3). CP treatment alone resulted in a VDT of 18.7 days and GD of 21.8 days. Ot or Vc pretreatment produced a slightly higher, though not significant, VDT (Ot + CP: 21.7 days; Vc + CP: 20.8 days) and almost equal GD (Ot + CP: 22.5 days; Vc + CP: 22.9 days) as CP alone.

In the case of fibrosarcoma (Table 3), RT alone, Ot + RT and Vc + RT resulted in almost similar CR, 50% in the RT and Ot + RT groups and 40% in Vc + RT, and all of these survived up to 120 days. Another 10% survived with tumour in the Ot + RT group, giving a total survival of 60% in this group (Table 2). Control tumours had VDT of 1.7 days. All the treatments significantly increased the VDT to more than 20 days and GD to 30 days, giving almost similar values for the RT alone and Ot/Vc + Rt groups.

CP as well as Ot/Vc + CP treatments produced 100% CR. While 90% of the animals survived at 120 days without tumour recurrence in the CP-alone group, the tumour-free survival in both Ot + CP and Vc + CP groups was 100%.

Table 1. Response of mouse fibrosarcoma to OE and different anti-cancer treatments – lung colony assay

Treatment group	No. of cells injected	No. of colonies/10^4 cells injected
Control	10,000	19.68 ± 0.62
RT (30 Gy)	15,000	13.38 ± 0.88[c,f]
WA (40 mg/kg)	10,000	16.88 ± 1.22[b]
OE (50 mg/kg)	10,000	19.2 0 ± 0.57
CDDP (5 mg/kg)	15,000	13.68 ± 1.12
WA + RT	20,000	10.44 ± 0.99[d,g]
OE + RT	20,000	13.68 ± 0.78
OE + CDDP	15,000	13.28 ± 1.10
OE + WA	10,000	16.06 ± 1.57
CDDP + RT	20,000	9.52 ± 1.01[d,m,x,z]
OE + WA + RT	20,000	10.98 ± 1.35[y]
OE + CDDP + RT	30,000	9.40 ± 1.20[x,z]

Student's *t* test.
[b]$P < 0.01$; [c]$P < 0.001$ compared to control; [d]$P < 0.05$ compared to RT; [f]$P < 0.05$; [g]$P < 0.01$ compared to WA; [m]$P < 0.05$ compared to CDDP; [x]$P < 0.05$ compared to OE + RT; [y]$P < 0.05$ compared to OE + WA; [z]$P < 0.05$ compared to OE + CDDP.

Table 2. Response of mouse melanoma to RT and CP with or without Ot/Vc pretreatment

Treatment	VDT (days)	GD (days)	CR (%)	Survival (%)
Control	2.11 ± 0.37	–	0.00	00.00
DDW + RT	12.94 ± 1.28[c]	18.87 ± 2.12	0.00	60.00
Ot + RT	14.06 ± 1.10[c]	20.40 ± 1.44	0.00	50.00
Vc + RT	13.29 ± 1.10[c]	21.70 ± 1.31	0.00	60.00
DDW + CP	18.75 ± 1.57[c]	21.78 ± 1.46	0.00	40.00
Ot + CP	21.70 ± 2.01[c]	22.46 ± 1.93	20.00	50.00
Vc + CP	20.84 ± 1.09[c]	22.89 ± 1.76	20.00	40.00

[c]$P < 0.001$ compared to control; RT, 30 Gy; CP, 300 mg/kg body wt.

Table 3. Response of mouse fibrosarcoma to radiation and cyclophosphamide with or without Ot/Vc pretreatment

Treatment	VDT (days)	GD (days)	CR (%)	Survival (%)
Control	1.69 ± 0.08	–	00.00	00.00
DDW + RT	23.97 ± 1.48[c]	30.21 ± 1.69	50.00	50.00
Ot + RT	22.74 ± 2.09[c]	31.68 ± 1.94	50.00	60.00
Vc + RT	23.96 ± 1.27[c]	29.93 ± 1.81	40.00	50.00
DDW + CP	ND	ND	100.00	90.00
Ot + CP	ND	ND	100.00	100.00
Vc + CP	ND	ND	100.00	100.00

ND, Not done as all tumours showed complete regression and there was no regrowth during the 120 days of observation. Other explanations as in Table 2.

The Ocimum leaf extract and its flavonoids orientin and vicenin have been shown to give significant protection against radiation lethality[1,2] and clastogenicity[3–5] in mice. The flavonoids have also been found to protect cultured human lymphocytes against micronuclei induction by radiation[16] and mouse bone-marrow chromosomes against cyclophosphamide toxicity[17]. These findings establish their normal tissue protective efficacy. While using a drug for normal-tissue protection in cancer therapy, it is highly desirable that the drug should give significant protection to normal tissues with no or minimal protection to tumours. The present results show that neither the Ocimum leaf extract nor its flavonoids gave any protection to the two transplantable mouse tumours tested in this study, against radiation or chemotherapeutic drug toxicity. On the contrary, the flavonoids increased the response of melanoma to CP, as indicated by the higher CR% and VDT in the Ot/Vc pretreated groups. This suggests an additional advantage in using these compounds with CP chemotherapy.

An ideal normal-tissue protector for human applications should be effective orally and should be non-toxic at the effective dose. Our earlier studies have shown that oral administration of the extract and the flavonoids was also effective in giving significant protection against radiation lethality in mice[1,2]. The optimum effective doses of the extract and the flavonoids are well below their toxic doses (< 1/100 LD_{50} of the extract[1]; even less in the case of Ot and Vc[2]). Thus, these products appear to have all the qualities needed for an ideal protector, even though protection against lethal irradiation (DMF = < 1.5 at the optimum dose) is not as high as that given by the protective aminothiol, WR-2721 (DMF = 2.7)[18]. However, higher protection factors have been obtained by OE[3] and Ot and Vc[19] for bone-marrow protection against clinically relevant sub-lethal radiation doses. The present data also suggest that, in addition to the normal-tissue protection, Ot and Vc may even exert a potentiating effect on the tumouricidal activity of CP.

The mechanism of this preferential normal-tissue protection is not understood at present. The high hydrophilicity of the extract and the flavonoids may have an important role in facilitating easy access of the protectors to the normal cells, while absorption in the solid tumours may not be as efficient. A similar phenomenon has been reported[20] to be a key factor in the preferential normal-tissue protection by the phosphorthioate protector WR-2721.

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On the decreasing frequency of monsoon depressions over the Indian region

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Studies on the occurrence of monsoon depressions over a long period such as 1889 to 2002 show a significant decrease of their seasonal frequencies. Also, examination of monthly frequencies indicates that the decreasing trend was maximum in July followed by August and September. There is no trend in the month of June. Therefore, an attempt has been made in the present study, to examine whether any such variability also exists in the dynamical parameters representing the monsoon circulation such as horizontal and vertical wind shears, mean sea-level pressure (MSLP), middle level temperature, moisture, sea surface temperature (SST) and outgoing longwave radiation over the Bay of Bengal. NCEP/NCAR reanalysed data available for the period 1948–98 have been used for this study. Results show similar decrease in the horizontal and vertical wind shears of the mean monsoon flow over India as well as over the Bay of Bengal and decrease in the moisture and convection over the Bay area. There are corresponding increasing tendencies in the MSLP, temperature at 500 hPa and SST over the Bay of Bengal. Thus, in the present study, the decreasing trend in the number of monsoon depressions has been supported by the fact that the atmospheric dynamical parameters which favour formation of monsoon depressions have weakened in recent years.

AMONGST all the monsoonal weather systems, monsoon depressions are recognized as the main rainfall-producing synoptic weather systems over India. Intraseasonal and interannual variations[1] of monsoon depressions and their effect[2–4] on intraseasonal and interannual variation of Indian summer monsoon rainfall (ISMR) have been discussed by many authors. In addition to the interannual variation, ISMR exhibits strong interdecadal variations[5–10]. Characteristics of the monsoon disturbances and their relationship with ISMR in longer timescales such as decadal to 30-years, have also been examined[11]. These studies show the existence of dominant interdecadal shifts in the characteristics of monsoon disturbances and their relationship with ISMR. Results also indicate significant decrease in the frequencies of occurrence of monsoon depressions. Figure 1 *a* shows the 11-year running means of frequency anomalies of cyclonic storms and depressions over the Indian region in the summer monsoon season for the period 1889–2002. The decreasing trend since the seventies is well marked. Such decreasing trend in the frequency of occurrence of monsoon depressions over the Indian region is so predominant in recent years that their average seasonal frequency has come down to 2 or 3 in the most recent decade of 1993–2002, from its long-period average value of 7 to 8. It may be noted that for the first time in the last 115 years of data available with India Meteorological Department (IMD), not a single depression or cyclonic storm formed over the Indian region, including the Bay of Bengal and Arabian Sea during the monsoon season of 2002.

Based on observed data from IMD, the occurrence of low-pressure areas (LOPARS) is also examined and their 11-year running means are shown in Figure 1 *b*. Interestingly, the study shows that the number of LOPARS has increased significantly from 1970s to the recent decade. Since the number of LOPARS shows increasing tendency and that of depressions and cyclonic storms indicates decreasing trend, it may be inferred that the dynamical parameters of the monsoon flow are not favourable for the intensification of lows into depressions and cyclonic storms.

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