



Tumour promoter activity in Malaysian Euphorbiaceae

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Summary Herbal medication has been practised by the rural Malaysian Malays for a long time. However, the long-term side-effects have never been studied. In the present study, 48 species of Euphorbiaceae were screened for tumour-promoter activity by means of an *in vitro* assay using a human lymphoblastoid cell line harbouring the Epstein–Barr virus (EBV) genome. Twenty-seven per cent (13 out of 48) of the species tested were found to be positive, and in four species, namely *Breynia coronata* Hk.f., *Codiaeum variegatum* (L.) Bl, *Euphorbia atoto* and *Excoecaria agallocha*, EBV-inducing activity was observed when the plant extracts were tested at low concentrations of between 0.2 and 1.2 µg ml⁻¹ in cell culture. This observation warrants attention from the regular users of these plants because regular use of plants with tumour-promoting activity could well be an aetiological factor for the promotion of tumours among rural Malaysian Malays.

Keywords: tumour-promoter activity; Euphorbiaceae; EBV early antigen induction

Epidemiological studies reveal a strong circumstantial link between plants possessing tumour-promoting principles and the increased incidence of human tumours in certain geographic regions of the world. Hirayama and Ito (1981) indicated that there is a strong circumstantial link between the high incidence of nasopharyngeal carcinoma (NPC) in the southern regions of China and the distribution of Euphorbiaceae and Thymelaeaceae plants. Among these plants, extracts from *Croton tiglium*, *Euphorbia lathyris*, *Aleurites fordii*, *Jatropha curcas*, *Euphorbia antiquorum*, *E. milii*, *E. pekinensis*, *E. kansui* and *Daphne odora* all exert Epstein–Barr virus (EBV)-activating capacity. The active component of the classic tumour promoter of croton oil from *Croton tiglium* L was purified and its chemical structure defined as 12-*O*-tetradecanoyl phorbol-13-acetate (TPA) (Hecker, 1968).

A similar study also indicated a causal link between the regular intake of phorbol esters contained in 'Walensalii tea' prepared from *Croton flaven* L. (Euphorbiaceae) and the high incidence of oesophageal cancer in Curacao Island of the Caribbean (Hecker, 1987).

Burkitt lymphoma (BL), an EBV-associated non-Hodgkin's malignant lymphoma, is endemic in an area of Africa known as the lymphoma belt. In East Africa (Kenya, Uganda and Tanzania), where BL is endemic, over 90 species of Euphorbiaceae plants are reported to be currently used as common folk remedies (Kokwaro, 1979). EBV-activating capacity has been demonstrated in extracts of some of these plants (Ito, 1986).

EBV-activating capacity has also been reported in 11 out of the 12 species of the Euphorbiaceae family commonly grown in the Cameroon, one of the endemic areas of BL (Ohigashi *et al.*, 1985). In Malawi, *E. tirucalli* and other EBV-activating plants were found significantly more often in homes of BL patients than in those of controls (Van den Bosch *et al.*, 1993). Recently, *E. tirucalli* has been reported to induce the characteristic 8:14 translocation of endemic BL in EBV-infected lymphoblastic cell lines *in vitro* (Aya *et al.*, 1991).

In Malaysia, herbal medicaments are widely used by the rural Malays. Several parts of the different plants are used. Dried parts of the plants such as roots and barks are usually boiled and the decoction taken internally. Fresh specimens are usually squashed and applied externally as a poultice.

Many of the indigenous plants used are also from the Euphorbiaceae and Thymelaeaceae families. NPC, which is

associated with EBV, is also common among the Malaysian Malays, although the prevalence is lower than in the Malaysian Chinese (Armstrong *et al.*, 1979; Yadav *et al.*, 1985). It would be interesting to study the incidence of NPC in relation to the use of these plants. However, in this study, we only screen the members of the Euphorbiaceae used by herbalists for their tumour-promoting activity.

It has now been established that latent EBV can be induced and activated from genome-carrying human lymphoblastoid cells *in vitro* by treatment with tumour-promoting phorbol and other diterpene esters (zur Hausen *et al.*, 1979; Ito *et al.*, 1981a), and indole alkaloid (Luka *et al.*, 1979; Ito *et al.*, 1981b; Yamamoto *et al.*, 1981). In the present study, a short-term *in vitro* assay for detecting tumour-promoting activity using the activation of EBV antigen expression in EBV genome-carrying human lymphoblastoid cells (Raji) has been used as the screening assay (Ito *et al.*, 1981b). This assay system is rapid and efficient for detecting EBV-active principles in the environment (Ito *et al.*, 1981a,c).

It is also noteworthy that such EBV-activating principles can be considered to possess tumour-promoting capacity (Ito *et al.*, 1981a). The assay can thus be used as a screening test in the search for promoter substances in nature.

Materials and methods

Collection of plants

The Euphorbiaceae species were collected from various places in Malaysia. Some of the plants were collected from the University of Malaya botanical garden, Rimba Ilmu. The rest of the plants were collected during field trips conducted in the Malaysian states of Selangor, Pahang, Kedah and Kelantan. The list of plants collected is shown in Table I.

Preparation of crude extract

Crude ether extracts of dried, powdered plant material were prepared as previously described (Yadav *et al.*, 1989). Ten grams of the powdered material was extracted with ether (10 ml) at room temperature for 48 h. After evaporating the solvent, the resultant residue was weighed and dissolved in dimethyl sulphoxide (DMSO) (Sigma) as a stock solution of 20 mg ml⁻¹ and kept at -20°C until use.

EBV early antigen induction in Raji cells

The technique used was as previously described (Ito *et al.*, 1981a; Yadav *et al.*, 1989). Briefly, rapidly growing human

Table I List of botanical names of Euphorbiaceae species screened for Epstein-Barr virus early antigen induction in Raji cells

<i>Acalypha hispida</i> Burm. f	<i>Elateriospermum tapos</i>
<i>A. indica</i> L	<i>Euphorbia atoto</i>
<i>A. siamensis</i> Oliv ex Gage	<i>E. hirta</i> L
<i>A. wilkensis</i> Moorea	<i>E. tirucalli</i> L
<i>A. wilkensis</i> Macafeana	<i>E. pulcherrima</i> Willd
<i>Agrostistachys longifolia</i>	<i>E. splendens</i>
<i>A. sessifolia</i>	<i>Excoecaria agallocha</i>
<i>Antidesma cupidatum</i>	<i>Glochidion brunneum</i>
<i>A. pendulum</i>	<i>G. lucidum</i>
<i>A. salicinum</i> Ridl	<i>G. macrostigma</i>
<i>Aparusa prainiana</i> King ex Gage	<i>Homalanthus populneus</i>
<i>Baccaurea dukis</i> Muell Arg	<i>Jatropha gossypifolia</i> L
<i>B. lanceolata</i> (Mig)	<i>J. podagrica</i>
<i>B. parviflora</i> (MA) MA	<i>Macaranga heynei</i>
<i>B. scortechinii</i>	<i>M. teccurrata</i>
<i>Breynia coronata</i>	<i>M. species</i>
<i>Cheatocarpus castanocarpus</i>	<i>Pedilanthus tithymaloides</i> (L) Poitt
<i>Chephalomappa mallotica</i> JJ Smith	<i>Phyllanthus frodosus</i> Wall
<i>Cicca acadia</i>	<i>P. niruri</i>
<i>C. accidia</i> Merr	<i>P. reticulatus</i> Poirr
<i>Coedium variegatum</i> (L) Bl	<i>Portulaca oleraceae</i>
<i>Croton argyratus</i> Bl	<i>Suregada angustifolia</i> (MA) Air Saw.
<i>C. candalus</i>	<i>Sebastiania chamaelea</i> (L) MA

lymphoblastoid Raji cells were incubated with plants extracts in the presence of 4 mM sodium butyrate. The cells were incubated for 72 h in a humidified incubator at 37°C with 5% carbon dioxide in air. The positive control consisted of Raji cells treated with optimal concentration (10 ng ml⁻¹) of TPA (Sigma) and 4 mM *n*-butyrate (Sigma). The negative control consisted of untreated culture media. After 72 h, the cells were harvested and checked for the presence of EBV early antigen by means of the indirect immunofluorescence assay. Nasopharyngeal carcinoma EBV-positive sera were used to detect the presence of EBV early antigen. The assay was repeated once in order to obtain consistent results.

Results

Twenty-seven per cent (13 out of 48) of the Euphorbiaceae screened were found to be positive for tumour-promoting activity when tested at concentrations of 10–40 µg ml⁻¹ cell culture medium (see Table II). Crude extract of four plants, namely *Breynia coronata* Hk.f., *Codiaeum variegatum* (L) Bl, *Euphorbia atoto* and *Excoecaria agallocha*, demonstrated EBV-induced activity even when tested at lower concentrations of between 0.2 and 1.2 µg ml⁻¹ cell culture. Relative to

Table II List of Euphorbiaceae species positive for Epstein-Barr virus early antigen (EA) induction in Raji cells

Botanical name	Medicinal uses	Plant part tested	Concentration of crude ether extract tested µg ml ⁻¹					
			0.2	0.6	1.2	10.0	20.0	30.0
<i>Breynia coronata</i> Hk f	Not documented	Leaf	-	-	+	+	+	+
<i>Coedium variegatum</i> (L) Bl	(a) Pounded root is rubbed into syphilitic sores ^a	Leaf	-	±	±	+	+	+
	(b) Leaves are used to poultice the abdomen of children with urinary problems ^a							
<i>Croton argyratus</i> Bl	(a) A decoction of the leaves is used as a post-partum protective medicine and a treatment for colic or diarrhoea ^b	Leaf	-	-	-	+	+	+
	(b) Leaves prescribed in medicinal bath to treat fever and as an application for ulcers ^a	Leaf						
<i>Euphorbia atoto</i>	Not documented	Leaf	-	-	±	+	+	+
<i>E. hirta</i> L	(a) It is made into poultices for sores on legs caused by the marine worm. The latex is dropped into the eyes for conjunctivitis and ulcerated cornea ^a	Whole plant	-	-	-	+	+	+
	(b) It has a reputation as a remedy for bronchitis and asthma, and is slightly narcotic, hence helping patients to sleep. ^a							
<i>E. tirucalli</i> L	(a) Roots and stems are used as a poultice applied to ulceration of the nose, haemorrhoids, swellings of the nose and painful parts ^a	Stem	-	-	-	+	+	+
<i>E. splendens</i>	Not documented	Stem	-	-	-	+	+	+
<i>Excoecaria agallocha</i>	(a) The latex is used in some places as a caustic in the treatment of obstinate ulcer ^b	Stem	+	+	+	+	+	+
	(b) A little of the bark chewed causes vomiting and purging and may be resorted to in constipation ^b	Leaf	-	-	-	+	+	+
<i>Homalanthus populneus</i>	(a) Oiled leaves are heated and applied to the stomach to treat fever ^b	Root	-	-	-	-	+	+
	(b) A little of the bark chewed causes vomiting and purging and may be resorted to in constipation ^b	Twig	-	-	-	+	+	+
<i>Jatropha gossypifolia</i>	(a) Seeds contain oil which is emetic and purgative ^b	Leaf	-	-	-	+	+	+
<i>J. podagrica</i>	Not documented	Seed	-	-	-	+	+	+
<i>Pedilanthus tithymaloides</i> (L) Poitt	(a) Applied to skin for leucoderma and for scorpion and centipede bites ^b	Stem	-	-	-	+	+	+
	(b) Leaves and stem also used as a meat tenderiser in cooking							
<i>Suregada angustifolia</i> (MA) Air Saw	Not documented	Leaf	-	-	-	+	+	+
		Stem	-	-	-	+	+	+

TPA (20 ng ml⁻¹) activated EBV EA in 30% of Raji cells. -, did not activate EBV EA in Raji cells; +, activated EBV EA in >30% of Raji cells; ±, activated EBV EA in <30% of Raji cells. ^aPerry (1980). ^bBurkill (1966).

the other plants. *Excoecaria agallocha* demonstrated the highest activity since the crude extract remained strongly positive even when tested at $0.2 \mu\text{g ml}^{-1}$ cell culture.

Discussion

Since plant diterpene esters in Euphorbiaceae and Thymelaeaceae plants have been found to exert EBV-inducing activity (Ito *et al.*, 1981b; Zeng *et al.*, 1983), only ether extracts were tested in the present study. However, the EBV-activating property has also been reported in methanol and water extracts of plants (Zeng *et al.*, 1983). It is to be hoped that other plant extracts will be tested in the future.

To date, no studies have been carried out to relate the use of indigenous plants with cancer in Malaysia. Therefore, the link between tumour promoters and cancers in Malaysia is unknown. From this study, it is disturbing to note the high prevalence of EBV-inducing activity in the species of Euphorbiaceae tested, especially since nasopharyngeal carcinoma, which is associated with EBV, is common among Malaysians (Armstrong *et al.*, 1979; Yadav *et al.*, 1985).

Since many of the EBV inducers are tumour promoters (Ito *et al.*, 1981b; Zeng *et al.*, 1983), the hypothesis that these positive plants are the aetiological factors for NPC is valid not only for NPC but also for other malignant tumours. Among the Malays, these plant extracts are either taken internally as a decoction or a concoction or used externally as a poultice. In both ways, there is direct contact between the plant extract and the skin or mucous membrane of the digestive tract. Plant extracts with EBV-activating capacity have been reported to retain their capacity to induce EBV complex in viral genome-carrying human lymphoblastoid cells (Raji) even after heat treatment at 120°C for 2 h or at 100°C for 12 h (Yanase and Ito, 1984). The medicinal plants with tumour-promoting activity could well be an important aetiological factor in the promotion of tumours among Malays who use these plants regularly in folk medication.

Detailed chemical studies on the positive EBV-activating plants identified in this study have not been carried out.

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- HOWEVER, the active compounds of *J. podagrica* and *J. gossypifolia* have been reported to be polyunsaturated esters of the tiglane-type diterpenoids 16-hydroxyphorbol and 12-deoxy-16-hydroxyphorbols respectively (Adolf *et al.*, 1984). Highly irritant and promoting principles of *E. tirucalli* have been characterised as 4-deoxyphorbol diesters (Kinghorn, 1979). The active tumour-promoting principle of *E. agallocha* has been shown to be the piscicidal daphnane-type orthoester 'excoecaria toxin', and the structures of the individual daphne-type irritant factors have been elucidated (Wikiya-chitra, 1985). Active principles of the other positive plants identified in this study have not been documented.
- EBV-activating principles have been documented to be present in *C. variegatum* (Ohigashi *et al.*, 1985), *E. hirta*, *E. splendens* and *P. tithymaloides* (Ito *et al.*, 1984). However, EBV-activating principles in *B. coronata*, *C. argyratus*, *E. atoto*, *H. populneus* and *S. angustifolia* have not been documented in the literature. *E. pulcherrima* did not exhibit EBV-activating principles and a similar observation has been reported previously (Ito *et al.*, 1984).
- The multifactorial aetiology of cancer is complex. However, the most important task of basic cancer research always was and still is the development of generally valid criteria for the detection and classification of environmental carcinogens. In Malaysia, good epidemiological studies regarding an association between the use of plants with tumour-promoting activity and the prevalence of cancer (tumours) are clearly needed. More plants should be screened for this activity, and further studies on tumour-promoting activity in Malaysian plants are necessary.

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