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Isolation of biologically active alkaloids from Korean mistletoe *Viscum album, coloratum*[1]

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Summary. Anticancer activity of certain highly cytotoxic alkaloids present in Korean mistletoe has been demonstrated in experimental animals. Unlike European mistletoe, no cytotoxic proteins were found in the Korean mistletoe.

Mistletoe plant belongs to genus *Viscum* which contains a variety of plants found all over the world. Mistletoe plant is a parasite and is found to grow on a variety of deciduous trees including apple, ash hawthorn, lime and acorn. The use of mistletoe for the treatment of human cancer had been suggested by Steiner[3]. Subsequent work using injectable preparations of European mistletoe 'Iscador' reported favorable effects against human malignancies[4-9].

The toxic properties and the toxic components of European mistletoe, *Viscum album* L., have been analyzed and described by Vester and reviewed by Evans and Preece[10]. Vester et al.[11-16] used extract of European mistletoe to demonstrate its anticancer activity against cultures of sarcoma 180 and HeLa cells. The antitumor activity of mistletoe extracts against sarcoma 180 in Swiss mice was also demonstrated. The most active component was present in a protein fraction whereas some activity was also found in protein free fractions. In these studies toxicity in vivo or cytotoxicity in vitro were not correlated with the carcinostatic (in vivo) effects of mistletoe. Samuelsson[17] has studied and isolated the toxic component of mistletoe *Phoradendron serotinum* growing on *Juglans hinsii* Jepson (collected in Martinez, California) and identified it as a protein (Phoratoxin) with mol wt of 13,000. Phoratoxin produced pharmacological effects which were similar to viscotoxin obtained from *Viscum album* L., but on weight basis Phoratoxin was 10 times less effective than viscotoxin. These proteins unlike normal chemotherapeutic agents were not immunosuppressive. There are literature[18] suggestions that some protein fractions may activate the immune competence of the host. Samuelsson has also described the isolation of Phoratoxin from mistletoe. *Phoradendron tomentosum* (DC) Engelin subsp. *macrophyllum* (Cockerell) Wiens[19]. The purified toxic protein was shown to have a mol. wt of 5000 and LD_{50} (lethal dose 50) in mice 0.57 mg/kg. The anticancer activities of the purified proteins have not been described. There is no literature describing toxic effects of alkaloidal components of European or Californian mistletoe. Here we wish to report the isolation of alkaloids from *Viscum album, coloratum* (a Korean mistletoe) and describe their anticancer activities.

Isolation of mistletoe alkaloids. Twigs and leaves from *Viscum album, coloratum* were ground and chopped in a blender and then extracted with aqueous acetic acid (2%) by continuous agitation of the suspension for 24, 48 and 72 h. At this time the suspension was filtered and the filtrate carefully lyophilized. Under these conditions a brown pow-

Table 1. Activity of mistletoe extract and mistletoe alkaloid fractions against in vitro cultures of leukemia L1210*

Concentration (μg/ml)	Growth inhibition (%)											
	Crude mistletoe extract	Alkaloid extract	Alkaloid fraction No.**									Iscador
			I	II	III	IV	V	VI	VII	VIII	IX	X	
810.00	100	100	–	–	–	–	–	–	–	–	–	–	92
230.00	100	100	–	–	–	–	–	–	–	–	–	–	44
23.00	23	100	100	100	100	100	41	98	44	40	45	41	6
2.30	20	21	17	100	78	86	–	26	–	–	–	–	–
0.46	6	–	7	95	18	51	–	12	–	–	–	–	–
0.23	–	–	14	63	19	33	–	26	–	–	–	–	–
0.09	–	–	7	10	9	11	–	7	–	–	–	–	–

* Suspension cultures of L1210 cells (6×10^4 cells/ml) were incubated at 37 °C in a CO_2 incubator for 48 h with indicated concentrations of various materials obtained from mistletoe. The results are expressed as number of cells counted in the drug treated dishes as compared to the untreated controls at the end of the incubation period.

** Fractions were obtained as chromatographically homogeneous components by preparative tlc of the mistletoe alkaloid extract.

dery residue could be isolated from the mistletoe. The residue was dissolved in 15 ml sterile phosphate buffered saline and the solutions used in subsequent biological evaluation studies as crude mistletoe extract. For the extraction of alkaloids, the crude extract was dissolved in water (pH 5) and the aqueous solution extracted with petroleum ether repeatedly. The aqueous layer was brought to pH 8.5 with $NaHCO_3$ and the solution was extracted with $CHCl_3$ several times. The $CHCl_3$ extract was filtered and evaporated under reduced pressure to obtain alkaloidal fraction (yield, 0.38%). The alkaloidal fraction was separated into 10 components by preparative TLC and subsequently by silica gel chromatography.

Biological evaluation. The crude mistletoe extract, the alkaloidal mixture and various alkaloidal fractions were screened for their biological activity against leukemia L1210 in vitro and against leukemia P388 in BDF_1 female mice in vivo.

The suspension cultures of leukemia L1210 cells were grown in RPM1-1640 media containing 10% fetal calf serum. The logarithmically growing cells were exposed to varying concentrations of the mistletoe extract for 48 h. Under these conditions mistletoe extract completely inhibited the growth of leukemia L1210 (table 1). At concentration of 230 µg/ml there was definite loss of cell number indicating cell death.

Table 1 shows the effect of alkaloid extract and various alkaloidal components against leukemia L1210 in vitro. Table 1 shows that alkaloidal fractions II and IV were most effective in inhibiting the growth of the leukemia (ED_{50}, 0.19 µg/ml and 0.45 µg/ml respectively). The crude mistletoe extract after the extraction of alkaloids still showed some residual activity against leukemia L1210 in vitro. However, after a pronase (obtained from Calbiochem) treatment which hydrolyzed proteins (table 2), the extract retained this activity indicating that the activity may be due to residual alkaloids and not an intact protein. Crude mistletoe extract was tested for its activity in vivo against leukemia P388 in BDF_1 mice. Mistletoe extract at a dose schedule (table 3) of 0.2 ml/mouse (qd, 1-7) caused a 29% increase in the life span of the leukemic mice. In preliminary studies mistletoe alkaloidal fractions (table 3) at dose of 135 mg/kg caused a 77% increase in the life span of the leukemic mice, 2/6 of the treated animals were cured.

The studies presented here demonstrate that in experimental animals Korean mistletoe extract has therapeutic anticancer effects and these effects, unlike European mistletoe, are due to the presence of certain highly cytotoxic alkaloids. The possibility of the presence of similar cytotoxic alkaloids in European mistletoe is being investigated in our laboratory[20].

Table 2. Activity of mistletoe extract against leukemia L1210 in vitro after pronase treatment*

Treatment	Cell No./ml	Inhibition (%)
Controls		
Phosphate buffer	1.30×10^5	-
Pronase	9.74×10^4	12
Mistletoe extract	5.33×10^3	100
Mistletoe extract after pronase treatment	5.20×10^3	100

* Mistletoe extract (0.81 g) containing 2.0 mg total protein was incubated at 37 °C with 2.25 proteolytic units (PU) of pronase in a 0.03 M phosphate buffer (pH 7.4, 10 ml) for 72 h. At the end of the incubation period the pronase was denatured by heating the solution at 100 °C for 10 min followed by removal of the coagulated proteins by centrifugation. The supernatant (0.5 ml) was added to logrithmically growing suspensions of leukemia L1210 cells (4.5 ml media containing 8.7×10^3 cells/ml). The growth inhibition was calculated after 48 h incubation of each tube.

Table 3. Activity of crude mistletoe extract and total alkaloidal fraction against leukemia P388 on BDF_1 (C57B1/6 × DBA/2) female mice

Treatment (i.p. injection)	Extremes (days)	Average survival (days)	Δ wt (g) (day 5)	40-day survivors	Increase in life span
Controls, 0.9% saline	(8-11)	9.55	+1.90	0/10	-
Mistletoe extract					
0.1 ml, qd (1-7)	(11-14)	11.50	+1.40	0/6	20
0.2 ml, qd (1-7)	(9-19)	12.34	+0.99	0/10	29
Controls, 0.9% saline	(11-12)	11.25	+0.60	0/8	-
Mistletoe alkaloids					
62.5 mg/kg, qd (1-7)	(9-12)	10.83	+1.50	0/6	−4
135 mg/kg, qd (1-7)	(11-36)	20.00	+1.00	2/6	77

* Groups of (6-10) animals (18-20 g) were inoculated with 1×10^6 viable leukemic cells (i.p.). Treatments were started 24 h post tumor implant. Results are expressed as percent increase in the life span of the treated animals as compared to the saline treated controls. National Cancer Institute (USA) protocol for screening natural products were followed.

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