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Antiviral Activity of Plant Extract Liv.52 in Mice Experimentally Infected with Semliki Forest Encephalitis Virus

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There are several reports in literature to show that plants may be effective sources of supply of antiviral agents. Kucera *et al.*[1], drew attention to antiviral activity of extract of lemon balm (*Melissa officianlis*) plant against at least four mammalian viruses tested in tissue culture models. Furusawa and Cuttings[2] tested 130 plant extracts against Columbia SK virus in experimentally infected animals (mice) and found two that protected animals from challenge infection.

Semliki forest virus (SFV), a RNA arbovirus originally isolated from mosquitoes by Smithburn and Haddow[3,4] has been extensively employed in recent years for production of experimental viral encephalitis[5,6] in laboratory animals (mice) and study of antiviral chemotherapy[1,7]. Development of orally active antiviral drugs against SFV would be of great interest particularly those that will act in concert with immunologically or therapeutically active virus inhibitory substances.

This paper reports results of tests we carried out in regard to anti-SFV activity in mouse model of Liv.52 (The Himalaya Drug Co.) which is a crude, herbal preparation extensively used in the indigenous (Ayurvedic) system of medicine in India and recently shown to have beneficial effect in treatment of viral infections in man[8,9].

Mice: 16 g mice of either sex were obtained from a stock of CDRI line of out bred Swiss mice.

Liv.52: Liv.52 is a brand name for a herbal preparation marketed by The Himalaya Drug Company and is a mixture of extracts from 18 herbaceous plants. Liv.52 sample was obtained powder form. Its use is recommended by its makers in patients wherein a correction of liver function is indicated such as in conditions like acute viral hepatitis[8]. The eighteen plants used by the manufacturers in preparation of Liv.52 are: *Capparis spinosa, Cichorium intybus, Solanum nigrum, Cassia occidentalis, Terminalia arjuna, Achillea millefolium, Tamarix gallica, Eclipta alba, Phyllanthus niruri, Berberis aristata, Raphanus sativus, Phyllanthus emblica, Plumbaga zeylanica, Boerhaavia diffusa, Tinospora cordifolia, Embelia ribes, Terminalia chebula* and *Fumaria officianlis.*

Virus: Semliki forest virus was originally obtained from ATCC, USA and had undergone over 300 mouse passages at CDRI. In mice inoculated by the subcutaneous (s.c.) route, the virus normally has an average incubation period of 3 to 6 days. Death of inoculated animals is preceded by sickness and paralysis of fore or hind legs or of both. Brain tissue from such infected mice reaches a virus titre of about $10.^{6}$

6MFA: 6 MFA, an interferon inducing antiviral substance and chemically a double stranded RNA in mixture with polysaccharide, was made available from stock at CDRI[11] and was employed for the purpose of comparison.

Liv.52 powder was weighed and suspended in normal saline at the rate of 10 mg/ml. Each volume (10 mg/ml) was orally administered to test animals by syringe, twice a day, morning and evening, starting before and continuing after challenge infection. Control animals received no Liv.52. Dosage of Liv.52 was varied from 30 to 100 mg per mouse (1.8 gm/kg to 6.0 gm/kg). Table 1 summarises the results that were obtained; they provide evidence that Liv.52, upon administration in large doses, protected a significant proportion (30%) of challenged infected mice.

Table 1: Administration of Liv.52 alone: Protection rate obtained against SFV in experimentally challenged infected mice

Group	Total mice	Treatment gm/kg	Administration (1 dose=10mg)	Survival (%)
1	10	1.875	Before challenge (1 dose) After (2 doses)	0
2	10	3.125	Before (5 doses)	0
3	10	3.125	Before (5 doses)	0
4	10	6.250	Before (5 doses) After (5 doses)	30

NB: Control untreated SFV (100 LD_{50}) inoculated mice showed 100 per cent mortality.

Table 2 summarises the results of tests performed with Liv.52 in association with interferon inducer. Data show that administration of Liv.52 in infected mice does not interfere with the mouse protective activity of Liv.52 is under investigation.

Table 2: Administration of Liv.52 in combination with interferon inducer: Effect of net protection from infection with SFV in experimentally inoculated mice

Group	Total mice	Treatment (gm/kg)	Administration (before or after challenge)	Net protection (Mortality)	Mean survival time (days)
(a)	10	Saline (i.p.) (Control)	1 ml	90	5.6
(b)	10	*6MFA (ip)	Before (1 dose)	20	9.0
(c)	10	6MFA + Liv.52 (3.125 mg)	Before (1 dose) + Before (5 doses)	10	12.0
(d)	10	6MFA + Liv.52 (6.250 mg)	Before (1 dose) + Before (5 doses) and After (5 doses)	0	20.0

*180 mg/kg body weight i.p. (intraperitoneal)

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