Animal Experimentation with Herbal Product Septilin: 
Increase in Average Survival Time of Mice Treated with Septilin in 
Presence of Interferon Inducer and Challenge Infected with Lethal Doses 
of Semliki Forest Virus

Gupta, B.M., Principal Investigator, Interferon Project (DST), 
Gupta, K.P., Junior Research Fellow, and Lata Dusre, Junior Research Fellow, 
Division of Virology, Central Drug Research Institute, Lucknow, India.

ABSTRACT
Septilin (The Himalaya Drug Co.) a crude mixtures of oleo gum resins and several plant 
extracts, has been found to be able in the presence of interferon inducer 6-MFA (Maheshwari 
et al., 1977; Gupta, 1978) to increase significantly the mean survival time of test mice 
challenge with semliki forest encephalitis virus.

INTRODUCTION
Lately there has been a return of interest in the study of the phenomenon of non-specific 
stimulation of body processes, particularly immune reactions against viral infections by 
treatment with exogenous substances and for protection against noxious stimuli (Kirchner et 
al. 1983; Farber and Glasgow, 1973). Plant saponins and lectins are the major substances that 
have been the most studied from this point of view. The mechanism of action of these plant 
products is poorly understood in most cases. Constituents of both Asiatic and Siberian 
ginseng (Panax ginseng and Eleutherococcus senticosus) have been found to increase adrenal 
capacity (Kim et al., 1970). Results of one careful study (Gupta et al., 1980) indicated that 
ginsenoside from Panax ginseng (Araliaceae) induces interferon production as could be 
judged by in vitro studies and also has been shown to augment natural killer (NK) and 
antibody dependent cytoxic (ADCT) activities in human peripheral blood lymphocytes. 
Further work suggested that in animals mice who were orally given ginseng in combination 
with interferon inducer (Singh et al., 1983) and later challenged with Semliki be enhanced, 
under conditions when the inducer alone gave significantly lower rate of protection. Lately, 
both Asiatic ginseng (P. ginseng) and Siberian ginseng (Eleuthero-coccus senticosus) have 
been found to increase radiation resistance of cultured mammalian cells through alteration of 

In our continuing programme of screening Indian plant products for occurrence in them 
of body defence stimulants we tested a product Septilin (The Himalaya Drug Co.) a crude 
mixture of oleo gum resin and several plant extracts. It has been found that in the presence of 
interferon inducer 6-MFA (Maheshwari et al., 1977), the mean survival time of the test mice 
challenged with Semliki forest virus is significantly increased by Septilin treatment.
MATERIALS AND METHODS
Septilin was given by The Himalaya Drug Co., Bombay in the form of dry powder and has the following composition:
Each Septilin tablet contains:
Balsamodendron mukul 0.162 g
Maharasnadi quath 65 mg
Exts.  Phyllanthus emblica 16 mg
        Tinospora cordifolia 49 mg
        Rubia cordifolia 32 mg
        Moringa pterygosperma 16 mg
        Pristimera indica 6 mg
        Shankh bhasma 32 mg

6-MFA (Interferon inducer) was made available from CDRI stock. It was obtained in the form of powder (Singh et al., 1981). Chemically 6-MFA is a polysaccharide (88% w/w) in mixture with nucleoprotein (12% w/w) of which the nucleic acid is double stranded RNA, the later being responsible for induction of interferon in vertebrate hosts.

EXPERIMENTAL
Septilin powder (30 mg) was suspended in phosphate buffer saline (pH 7.2). The suspension was shaken vigorously for 20 min and kept at 40°C overnight. The suspension was further shaken at 50°C in water bath for 30 min. The supernatant was removed and chilled acetone (1:1) was added. After 4 hrs the mixture was centrifuged. The precipitate was collected and suspended in buffer saline. This was used as such for administration to test animals. Animals (mice, 20 gm) obtained from CDRI random bred Swiss stock, were given Septilin preparation at the rate of 0.1 ml per dose orally. The number of doses given is shown under ‘treatment column’ and in the footnote under Table 1.

<table>
<thead>
<tr>
<th>Schedule of treatment</th>
<th>Mice</th>
<th>Survival (%)</th>
<th>Average survival time</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Phosphate buffer saline [Control]</td>
<td>10</td>
<td>0.0</td>
<td>5.7</td>
</tr>
<tr>
<td>2. [a] Septilin [P1, T0] Plus 6-MFA [0.1 ml]</td>
<td>10</td>
<td>10.0</td>
<td>6.2</td>
</tr>
<tr>
<td>3. Septilin [P11, T7] @ 0.1 ml</td>
<td>8</td>
<td>25.0</td>
<td>8.5</td>
</tr>
<tr>
<td>4. 6-MFA [0.1 ml]</td>
<td>10</td>
<td>10.0</td>
<td>6.3</td>
</tr>
<tr>
<td>5. [a] Septilin [P11, T7] Plus 6-MFA [0.1 ml]</td>
<td>10</td>
<td>50.0</td>
<td>12.6</td>
</tr>
</tbody>
</table>

[a] = Oral administration;  [b] = Subeffective dose [low dose];  [c] = [P1, T0] = 0.2 mg;  [d] = [P11, T7] = 3.6 mg
P stands for prophylactic and T stands for therapeutic.
For priming the animal with interferon inducer (6-MFA), test animals were invariably injected by i.p. route at the rate of 0.1 ml (0.3 mg) per animal 24 hours prior to virus challenge.

The virus (Semliki forest virus) when injected subcutaneously; produces encephalitis and death of animals in 4 to 10 days depending upon the size of the inoculum. The test dose of the virus was so chosen as to produce just 100% death in the aforesaid time of contact (incubation) with mice.

Observations were taken daily morning and evening for a period of 20 days following challenge infection. Results in terms of survival rate (%) and average survival time are recorded in the table presented.

RESULTS AND DISCUSSIONS
There is a dose dependent response in mice to Septilin (Table 1) when total doses were increased from one to eighteen (row 2 and row 5) in presence of constant amount of 6-MFA. Secondly, the average survival time of test animals is also increased by increasing the doses of Septilin in combination with constant dose of 6-MFA (6.2 days to 12.6 days). Two parameters, percent survival and average survival time, taken together, are thus enhanced and this could be interpreted as being due to Septilin extract. The chemical nature of the Septilin extract is not fully known and is under investigation. The mechanism(s) by which Septilin extract enhances mouse response (antibody, cell-mediated immunity and interferon) are also under investigation.

REFERENCES