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IN VITRO ANTIVIRAL POTENTIAL OF CERTAIN INDIAN MEDICINAL PLANTS AGAINST POLIO VIRUS

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SUMMARY

Four Indian medicinal plants viz tulsi (*Ocimum sanctum* Linn), neem (*Azaderichta indica* Juss), lehson (*Allium sativum* Linn) and adrak (*Zingiber officinale* A. Ross) were assessed for their in vitro potential to inhibit poliovirus type 3 replication using VERO cells. Results showed that a non-toxic range of 22.5 to 175 mg/ml) uconcentration of ethanolic extract of tulsi leaves inhibited poliovirus type 3 replication as compared to 225 to 1.75 mg per ml pf aqueous extract. Percentage inhibition of virus determined by virus inhibition assay for ethanolic and aqueous extracts of tulsi leaves was found 99.90% and 99.65% respectively. Aqueous extracts as well as ethanolic extracts of neem, lahson and adrak did not show significant viral inhibition.

INTRODUCTION

Poliomyelitis caused by polio virus is a major public health problem in developing countries (Minor et al 1990), Several plants namely neem, tea etc have been evaluated for their in vitro antiviral potential against polio virus (Vanden Berghe et al 1978, John and Mukundan 1978,1979 & Amoros et al 1992). However, there is very scanty information available for common domestic plants such as tulsi, lahson, neem and with regard to their antiviral activity against polio virus. Though tulsi has been reported to inhibit vaccinia and Ranikhet disease virus (Dhar et al 1968).

In the present study, in vitro antiviral activity of tulsi, neem, lahson and adrak was evaluated. The protective efficacy of different plant extracts was monitored by inhibition of poliovirus type 3 replication as indicated by reduction of cytopathic effect (CPE) in virus inhibition assay and determination of percentage of viable cells by neutral red dye uptake.

MATERIALS AND METHODS

Aqueous and ethanolic extracts

For preparation of aqueous extracts leaves were cut in small pieces, washed, air dried, powdered and dissolved in phosphate buffer saline (PBS), centrifuged at 500xg and filtered through 0.22 um pore size membrane filter. In case of adrak and lahson bulbs were peeled off, cloves cut into piecesand dried. Further same procedure was followed as described above followed thereafter.

Ethanolic extract of tulsi leaves was prepared as per the method described by Van Deb Berghe et al 1978. Wherein leaves were dissolved in absolute alcohol in a ratio of 1:3 prefiltered, concentrated, dissolved in PBS, diluted with minimum essential medium (MEM) filtered through 0,22 membrane filter and stored at -20. Adrak and lahson ethanolic extracts were prepared as per the method described by Khare (1995).

Cell cultures and virus

Vero cell lines (African green monmey kidney cells) were maintained by regular sub-culturing. Poliovirus propagated in vero cell cultures. Tissue culture infective dose 50% (TCID50) was determined according to Reed and Muench 1938 formula.

Cytotoxicity testing

Toxicity to vero cells caused by plants extracts was determined by following the protocol of Van Den Berghe et al (1978). All concentrations of plant material showing toxicity to cells were excluded and concentrations below non toxic dose (MNTD) were employeed for antiviral screening.

Virus inhibition assay

Serial 10 fold dilutions of virus were prepared and two fold dilution of plants extracts were prepared. Tulsi leave extracts aqueous (225 to 1.75) and ethanolic (22.5 to 0.175 neem leaves aqueous extract (0.25 to 0.031), adrak (0.8 to 0.062) and lahson aqueous extracts as (100 to 0.75) and mixed in equal proportion. 100ul of mixture were added in wells of MT plates.

Control wells contained healthy cells, virus and plant extracts. CPE was monitored after 24 hours.

Cell viability determination

Percentage of cell viability was determined by neutral red dye uptake method of Boren Freund et al (1988). In short, after appearing CPE, cells were fixed with 4,5%gluteraldehyde, washed and stained with 1% neutral red.

Subsequently, stained monolayer was washed and treated with).5% acetic acid and then observed colorimetrically by recording OD at 570 nm.

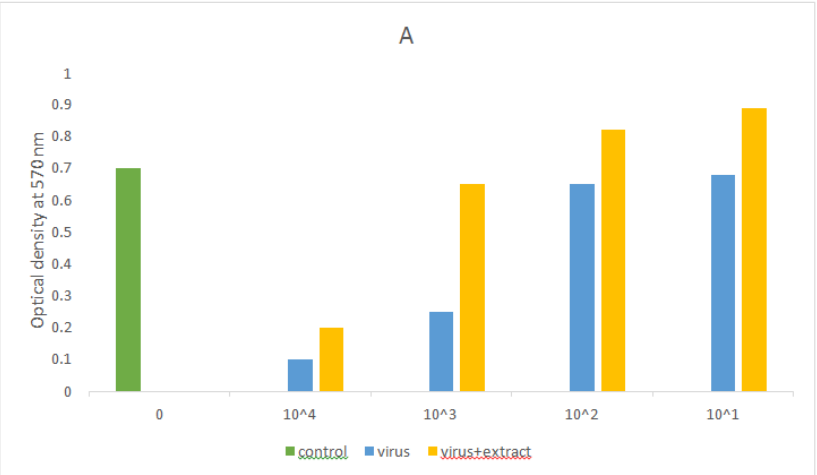
RESULTS AND DISCUSSIONS

Table-1 shows the spectrum of in vitro efficacy of different plant extracts against polio virus type 3. As evident maximum percentage inhibition of the virus by ethanolic extract of tulsi leaves at effective concentration (22,5 to 0.175 mg/ml) as determined as 99.90%.

Table 1: *In vitro* inhibitory effect of different plant extracts on p.

Name & part of the plant	Extract Prepared	Range of non-toxic effective conc. (mg/ml)	Inefectivity titre of virus (TCID50/ml Log10)		Percentage Inhibition
			Virus control	After incubation with plant extract	
Tulsi Leaves	Ethanolic	22. 5- 0.1 75	5.0	2.0	99. 90
	Aqueous	22 5- 1.7 5	5.0	2.0	99. 68
Neem Leaves	Aqueous	0.25-0.031	5.0	4.0	90.00
Adrak Rhizome	Ethanolic	8-0.062	5.0	4.0	90.00
Lahson Bulbs	Aqueous	100-0.75	5.0	4.5	68.38

Assessment of antiviral activity of plants against polio virus type 3.



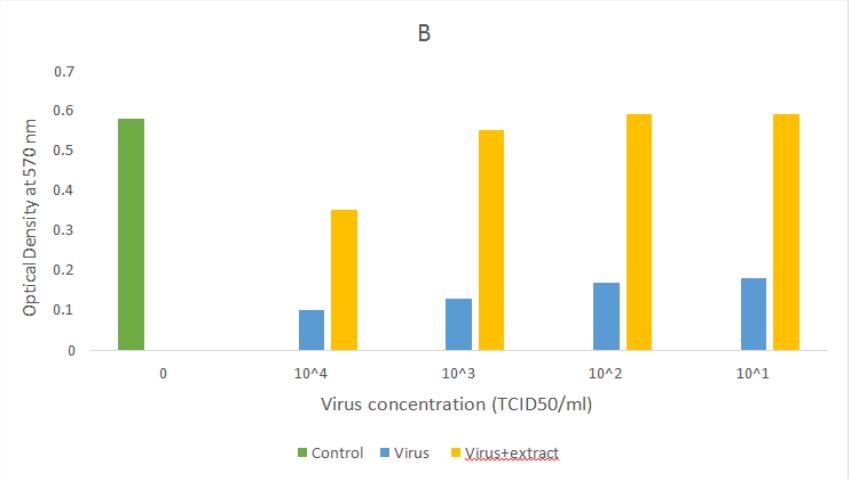


Fig. 1: Neutral red dye uptake study depicting the protective efficacy of tulsi leaves extracts against Poliovirus type 3 in non-toxic as well as effective range of concentration. (A) aqueous extract. (Effective conc. 225 mg/ml to 1.75 mg/ml) (B) ethanolic extract (22.5 mg/ml to 0.175 mg/ml).

The original infectivity titre of the virus was 5.0 TCID50, which showed a reduction to 2.0 TCID50 /ml after the incubation with different concentrations of ethanolic extract. On the otherhand aqueous extract of tulsi indicated relatively lesser percentage inhibition of 99.68% at a tenfold higher range of effective concentrations of tulsi leaves (225 to 1.75 mg/ml) and the titre was reduced to 2.5 TCID50 /ml).

Percentage inhibition of polio virus type 3, at the non toxic effective concentrations of both neem leaves and GEE was found to be 90% which is insignificant. Reduction in virus infectivity titre was also not adequately pronounced as it got diminished only to the extent of 4.0 TCID50/ml. Aqueous extract of lehson bulbs did not exhibit any activity as implied by an appreciably low percentage inhibition of 68.39%.

The results obtained with neutral red dye uptake studies indicating the cell viability and in turn the protective efficacy of tulsi leaves has been presented in Fig-1 A (aqueous) and B (ethonolic) extracts. The bar columns correspond to the average of OD values. obtained with the mixture having effective concentrations of plant extract and virus concentrations applied vis-a- vis identical concentrations of virus, plant extracts and healthy cell control. This was further substantiated by the total absence/reduction of viral specific CPE, as a result of protective efficacy of tulsi leaves. In case of tulsi aqueous extract (Fig. 1A) a dose dependent response was discernible even at the 10 TCID50 /ml titre of virus wherein a reduction of

CPE, was evident under micro- scopic observation, as compared to +++ CPE elicited by virus control. The protective efficacy of ethanolic extract of tulsi leaves has been depicted in Fig 1 (B). The average of OD at effective non-toxic concentrations (22.5 to 0.175 mg/ml) was found to be higher vis-a-vis virus control at 10 and 10 TCID50. Even at 10 virus concentration and plant extract mixture proportionately higher cell viability was evi- dent as manifested by an OD value of 0.55 against 0.12 by virus alone.

Since extracts of neem leaves, lehson bulbs and adrak rhizomes did not indicate significant antiviral activity against Polio virus type-3 as expressed in terms of percentage inhibition of virus, their cell viability profiles are not being depicted.

Though the traditional application of plants under study in combating human ailments have been widely elucidated (Chopra, 1958), there is a paucity of information on their evaluation with regard to their antiviral activity.

Ethanolic and aqueous extracts of tulsi leaves indicated relatively elevated *in vitro* activity in cell culture system in suppressing polio virus type-3 replication as compared to other plant extracts studied. It was substantiated by significantly high percentage inhibition of the virus i.e. 99.68 and 99.90% with aqueous and ethanolic extracts reregard spectively. It has been elucidated that a per- centage inhibition of virus 90% may be considered as significant and those below 90% as insignificant (John and Mukundan, 1979). Accordingly other plant extracts evaluated under this study did not show remarkable *in vitro* potential against polio virus type-3. Cell viability as indicated by the neutral red dye up- take test and represented as OD values denotes the progression of cell recovery from a state of viral induced CPE and in consequence the protective efficacy of the plant as compared to virus control. It is evident that higher OD values have been encountered with the aqueous and ethanolic extracts of tulsi leaves alone at their respective effective range of non-toxic doses vis-a-vis relatively lower OD values pertaining to healthy vero cells. It implies that at these effective concentrations, where the toxicity to cells gets diminished, some triggering effect exist to boost the viability of cells resulting in an elevated OD value (Nangia et al., 1983).

The virus inhibition test has been frequently applied as a versatile assay procedure to monitor the efficacy of a given piant extract to prevent the virus induced CPE at nontoxic concentrations (John and Mukundan 1978, 1979, Farrea et al., 1993. Premnathan et al., 1996). In the present study the same procedure was followed for the evaluation of *in vitro*

activity of the aforesaid indigenous plant extracts.

It may therefore, be concluded based on preliminary *in vitro* assessment of certain indigenous plants that both ethanolic and aqueous extracts of tulsi at 22.5 to 0.175 and 225 to 1.75 mg/ml concentrations respectively are effective in inhibiting polio virus type-3 replication. However, the ethanolic extract exhibits significantly higher potential as compared to aqueous extract.

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