Short Communication

IN VITRO EXPERIMENTS WITH *CENTELLA ASIATICA* : INVESTIGATION TO ELUCIDATE THE EFFECT OF AN INDIGENOUSLY PREPARED POWDER OF THIS PLANT ON THE ACID - FASTNESS AND VIABILITY OF *M.TUBERCULOSIS*

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The herb Centella asiatica (Linn.), found throughout India, is acclaimed to have medicinal properties and has been used in leprosy patients from very early times. It is considered that the active compound of this herb, called asiaticoside, probably acts on the waxy covering of M.leprae. The in vitro effect of an indigenously produced dry powder of Centella asiatica (CA) on the acid-fastness and viability of M.tuberculosis was investigated in the present study. The results indicate that CA may not have any direct action on the acid-fastness or viabilty of M.tuberculosis H37Rv in vitro. Further studies using purified asiaticoside of the plant or in vivo studies are required.

INTRODUCTION

Centella asiatica (Linn.) urban syn., *Hydrocotyl asiatica* (Linn.) is a herb found throughout India. In the indigenous system of medicine and elsewhere, this herb has been used in leprosy patients from very early times and is widely acclaimed to have medicinal properties (Boiteau et al, 1949, Kakkar 1988). In 1938, Bontemps isolated from this plant a new glycoside called asiaticoside which was active against leprosy (Notes and News 1945). Boiteau and Grimes obtained a solution of this compound suitable for injection and considered that it acted by dissolving the waxy covering of *M. leprae* so that the bacillus became very fragile and was easily destroyed by the tissues or by some other drugs (News and Views 1945). This led to the thinking that it was possible that the compound may have similar effect on other organisms like *M. tuberculosis* which also have the waxy covering. Here we report a study

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carried out to investigate the effect of an indigenously produced d
powder of *Centella asiatica* (CA) on the acid-fastness and viability of
tuberculosis in vitro.

MATERIAL AND METHOD

CA stock solution was prepared by weighing 60 mg of the powder of
(Pharm Products Pvt Ltd., India) and dissolving it in 3 ml of M/15 phosp
buffer pH 7.0. This extract was passed through a 0.22 µm membrane f
(Millipore, USA) for sterilising it and to remove undissolved matter.
medium containing the equivalent of 25, 50, 100, 200, 500 and 1000 µg of
per ml was prepared by aseptically adding 12.5, 25, 50, 100, 250 and 500 µ
CA stock solution respectively, to 10 ml of Sauton's medium with Tween 8
McCartney bottles.

These bottles were given random numbers and appropriate volume of
log phase culture of H37Rv was inoculated into each bottle to give 10^5 bac
ml. Immediately after inoculation (O hr), from each of the bottles, smears w
made using 10 µl of culture with 10 µl of formol milk (Hilson & Elek 1957),
dried, fixed with methanol and stained by Ziehl-Neelsen method. Viable co
(VC) was also set up from each of the bottles on selective 7H11 medi
(Mitchison et al 1972). Smear was repeated at 24 hours after inoculation.
was repeated at one hour, six hours, 24 hours and one week after inoculation.

The total number of AFB in each smear was counted by examining e
smear under oil immersion using a grid pattern. The selective 7H11 pla
were read under a colony counter after four weeks of incubation at 37 °C.

RESULTS

The results of the investigation are presented in the Table I. It can be se
that there was no reduction in the number of AFB in any of the bottles w
different concentrations of CA indicating that, under the in vitro conditio
used, the powder of *Centella asiatica* had no effect on the acid-fastness of
tuberculosis.

In all the bottles, irrespective of the concentrations of CA present, th
was a nett increase in the log cfu/ml after incubation at 37 °C for one wee
indicating that, under the conditions used in the present investigation, CA h
no effect on the viability of *M. tuberculosis*.

DISCUSSION

The results of the investigation carried out to elucidate the effect of t
indigenously prepared powder of *Centella asiatica* show that CA did not ha
any effect on the acid-fastness or viability of *M. tuberculosis* in vitro.

Table I. Log AFB/ml and log cfu/ml of *M. tuberculosis* H37Rv in Sauton's medium with different concentrations of CA after incubation at 37 °C for different durations

Conc. of CA	Log AFB/ml at			Log cfu/ml at					
in medium	O hr	24 hr	Increase	O hr	1 hr	6 hr	24 hr	1wk	Increase
ug/ml	a	b	(b - mean a)	c				d	(d - mean c)
0	4.73	4.79	0.05	4.54	4.81	4.74	4.74	5.11	0.49*
25	4.84	4.97	0.23	4.65	4.65	4.65	4.77	5.26	0.64*
50	4.62	4.97	0.23	4.49	4.69	4.30	4.87	5.00	0.38*
100	4.88	5.07	0.33*	4.77	4.77	4.54	4.60	5.23	0.61*
200	4.55	5.23	0.49*	4.60	4.74	4.54	4.92	5.00	0.38*
500	4.85	4.87	0.13	4.69	4.97	4.54	4.90	4.95	0.33*
1000	4.74	4.90	0.16	4.60	4.87	4.47	4.90	4.90	0.28*
Mean	4.74			4.62					
SD	0.12			0.09					

* Increase in count is greater than 2 SDs above the 0 hour mean.

Eventhough Boiteau and Grimes (News and Views 1945) considered that the asiaticoside extracted from *Centella asiatica* probably acted by dissolving the waxy covering of *M.leprae*, it has been suggested later (Chowdhury & Ghosh 1974) that the asiaticoside probably exerted its action by inhibiting the formation of acid-mucopolysaccharide (AMP) in humans (Boris & Stevenson 1965, Sasaki 1970). It has been shown that AMP is essential for the growth of *M. leprae* (Matsuo & Skinsness 1974). Therefore, if asiaticoside acted on *M. leprae* by blocking the formation of AMP, its effect could probably be demonstrated only in vivo using *M. leprae* and not under the conditions of the present in vitro experiment.

In conclusion, while the present investigation carried out in vitro failed to show any effect of *Centella asiatica* on the acid-fastness or viability of *M.tuberculosis*, similar in vitro investigations, but using purified asiaticoside of the plant so that incorporation of known concentrations of the compound in the test medium would be possible. In vivo experiments are also required for further study of its effect on *M. tuberculosis*.

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REFERENCES

1. Boiteau P, Buzas A, Lederer E, Polonsky J 1949. Derivatives of *Centella asiatica* used against leprosy. **Nature 163**: 258.
2. Boris A, Stevenson RH 1965. The effect of some unsteroidal anti-inflammatory agents on carcinogen induced exudate formation. **Arch Int Phar 153**: 205.
3. Chowdhury S, Ghosh S 1974. Mandukapami in the treatment of leprosy - Preliminary report. **Bull Calcutta School of Trop Med** 22: 4-5.
4. Hilson GRF, Elek SD 1957. Intratesticular multiplication of ***Mycobacterium lepraemurium*** in normal and suramin treated animals. **Int J Lepr 25**: 380.
5. Kakkar KK 1988. Mandukapami - Medicinal uses and therapeutic efficacy. **Indian Drugs 26**: 92-96.
6. Matsuo E, Skinsness OK 1974. Acid-mucopolysaccharide metabolism in leprosy. **Int J Lepr** 42: 392.
7. Mitchison DA, Allen BW, Carrol L, Dickinson JM, et al 1972. A selective oleic acid albumin agar medium for tubercule bacilli. **J Med Microbiol 5**: 165-175.
8. News and Views 1945. Treatment of leprosy. **Nature 155**: 601.
9. Notes and News 1945. An advance in the treatment of leprosy. **Lancet I**: 357.
10. Sasaki S 1970. Effect of hormones on the cutaneous connective tissue. **Skin Res (Osaka) 12:** 125.