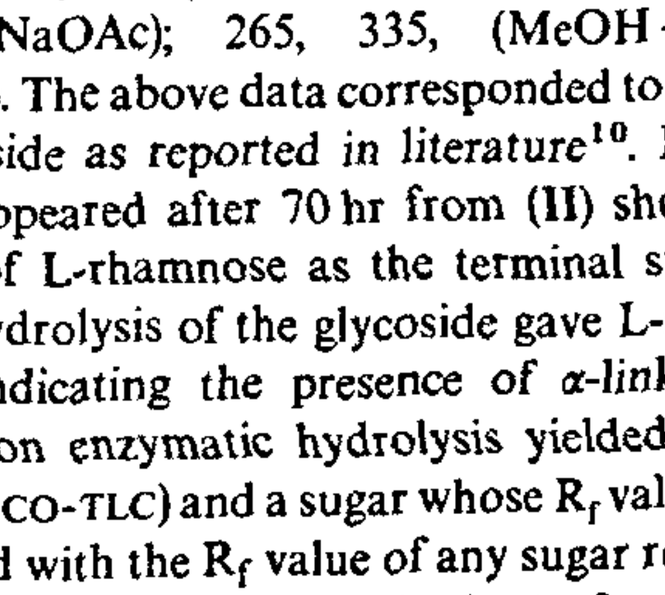
was finally confirmed with the authentic sample of apigenin (lit. mp, 347–48°, mmp and CO-TLC)[7]. Periodate oxidation[8(a,b)] consumed 3 moles of periodate with the production of 2 mol of the formic acid per 1 mol of the glycoside, suggesting the presence of a disaccharide in pyranose structure. The glycoside (200 mg) in Me_2CO (25 ml) was completely methylated with Me_2SO_4 (10 ml) K_2CO_3 (3 g) followed by acid-hydrolysis (7% H_2SO_4) afforded apigenin 4'-5-dimethyl ether, mp 160–62° (dec) [lit. mp, 160–63° (dec)] (mmp and CO-TLC)[9], 2,3,6-tri-O-methyl-D-glucose (R_G value, 0.83 *n*-BuOH : EtOH; H_2O, 5 : 1 : 4; and CO-PC)[4] and 2,3,4-tri-O-methyl-L-rhamnose (R_G value, 1.01 in *n*-BuOH : EtOH : H_2O, 5 : 1 : 4 and CO-PC)[4] respectively, showing the attachment of the sugars with 7-position of the aglycone part. The partial hydrolysis of the glycoside (200 mg) with H_2SO_4 (7% H_2SO_4, 25 ml) at room temperature, liberated L-rhamnose (CO-PC) after 50 hr and (II). The (II) had mp, 110–13° (dec), $C_{21}H_{20}O_{10}$; TLC; R_f 0.60 ($CHCl_3$: MeOH, 7 : 3) and 0.43 (Me_2CO : MeOH; 9 : 1); IR ν_{max}^{KBr}, 3400–3350 (br), 2900, 1660, 1600, 1500, 1450, 1375, 1380, 1220, 1060, 820, 660 and 610 cm^{-1}; UV λ_{max} 267, 335 (MeOH); 242 (sh), 268, 300 (sh), 380 (MeOH + NaOMe); 275, 298, 350 (MeOH + $AlCl_3$); 275, 297, 340, 370 (MeOH + $AlCl_3$ + HCl); 267, 385 (MeOH + NaOAc); 265, 335, (MeOH + NaOAc + H_3BO_3). The above data corresponded to apigenin-7-O-glucoside as reported in literature[10]. D-glucose (CO-PC) appeared after 70 hr from (II) showing the presence of L-rhamnose as the terminal sugar. The diastase hydrolysis of the glycoside gave L-rhamnose (CO-PC) indicating the presence of α-linkage. The glycoside on enzymatic hydrolysis yielded apigenin (mmp and CO-TLC) and a sugar whose R_f value did not correspond with the R_f value of any sugar reported in literature[4]. The hydrolysate, obtained after removing the apigenin on treatment with diastase solution afforded L-rhamnose (CO-PC) and D-glucose (CO-PC) confirming the presence of α-linkage between L-rhamnose and D-glucose and β-linkage between D-glucose and apigenin.

Hence, the glycoside was assigned a structure (I).



Structure 1

(I); R = rhamnosyl – glucoside
(II); R = glucose.

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PROTECTIVE EFFECT OF *MYCOBACTERIUM HABANA* IN MICE AGAINST INFECTION WITH INDIGENOUS STRAINS OF *M. TUBERCULOSIS*

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VIABLE *Bacillus Calmette Guerin* (BCG) is widely used in the prophylaxis of tuberculosis and is the only vaccine available for this purpose. However, there are several practical and theoretical objections for its use. These include the loss of sensitivity of tuberculin as a diagnostic and epidemiological tool and the suspected

protective efficacy of this vaccine against tuberculosis[1,2], which varies from 0 to 80 %. In a recent trial of this vaccine in South India, BCG has failed to protect against bacillary form of tuberculosis[3]. Several hypotheses have been put forward to explain the failure of the BCG vaccine. One of them is the prevalence of variants of *Mycobacterium tuberculosis*[4] which might not have been protected with the immunity generated by BCG. There is, therefore, a great need to select or to develop other immunogenic strain(s) of mycobacteria and or other eubacteria for vaccination. Our earlier work in this direction resulted in the identification of an atypical mycobacterium namely *M. habana* (TMC 5135) which was potentially immunogenic and provided protection against experimental tuberculosis and *M. ulcerans* infection in mice[5,6]. In this paper we present evidence to show that *M. habana* can protect against infection with several variants of the indigenous strains of *M. tuberculosis* in mice.

Freshly isolated and drug-sensitive strains of *M. tuberculosis* were obtained from Tuberculosis Research Centre, Madras, and their pathogenicity tested in mice. Depending upon the severity of infection in mice, these strains could be organised into three types *viz* highly virulent (velogenic) moderately virulent (mesogenic) and low virulent (lentogenic).

Seven groups of mice comprising of 10 animals in each, weighing 18–20 g, and bred in this Institute, were vaccinated with 1 mg wet weight (approximately 1×10^9 colony forming unit) of *M. habana* at 4 different sites subcutaneously and were challenged separately, with (0.5 mg/mouse) the seven indigenous strains of *M. tuberculosis*. The parameters of study included the weekly record of body weight, mortality,

Table 1 *Effect of vaccination with M. habana against indigenous strains of M. tuberculosis*[o]

Type and No. of challenge* strain	Group	No. of mice	Survival time in days			Statistical analysis†	
			Median	Mean ± S.E.	Range	t	P
Velogenic[x]	V**	9	57	53.8 ± 5.9	(34–84)	21 6	< 0.001
559182	U	10	20	20.9 ± 1.2	(15–26)		
Mesogenic	V	9	63	56.0 ± 7.7	(17–111)	8.2	< 0.001
560339	U	10	43	38.5 ± 5.8	(19–55)		
Mesogenic	V	8	114	105.0 ± 10.3	(51–140)	22.6	< 0.001
560712	U	10	32	46.0 ± 9.6	(17–86)		
Mesogenic	V	10	98	101.0 ± 8.6	(61–140)	20.5	< 0.001
560099	U	9	58	55.4 ± 6.8	(23–84)		
Mesogenic	V	8	106	115.0 ± 12.1	(71–150)	19.3	< 0.001
560235	U	10	64	61.2 ± 5.6	(38–89)		
Mesogenic	V	10	150	127.0 ± 12.1	(66–150)	27.0	< 0.001
560244	U	10	61	63.6 ± 5.43	(32–78)		
Lentogenic	V	10	114	130.8 ± 7.6	(108.150)	17.9	< 0.001
559749	U	10	97	89.5 ± 8.0	(26–114)		

V-vaccinated; U-unvaccinated
* Challenge dose = 0.5 mg (wet weight)/animal intravenously.
** Vaccinated S.C. with *M. habana* (1 mg wet weight/animal) 21 days before challenge.
† Comparison made within vaccinated and control groups using student t test.
• Experiment terminated on day 150 of challenge.
x Strain numbers of Madras.

necropsy score and impression smear examination for the presence of acid fast bacilli in the visceral organs. Every animal after death was examined to confirm the specific cause. The typical lesions of the organs provided a reasonable assurance that the animals died due to tuberculosis. The experiment was terminated after 150 days of infection.

The results presented in table 1 show that *M. habana* afforded protection against all *M. tuberculosis* strains used. The main criteria of protection were considered to be prolongation of survival time of vaccinated animals *vis a vis* control animals after being challenged with *M. tuberculosis* strains. Unvaccinated control group of animals challenged with highly and moderately virulent strains started showing deterioration in their general appearance and their body weight dropped considerably as compared to vaccinated animals. These animals died earlier and their visceral organs showed more intense lesions. The impression smears of the organs from these unvaccinated animals had an abnormally large number of acid fast bacilli. A few animals which survived in vaccinated groups challenged with moderately pathogenic strains showed either no lesions or healed lesions on biopsy at 150 days post infection.

The data have been statistically analysed and the *P* values were highly significant. Results show that *M. habana* was able to protect in acute, subacute and chronic types of infection in mice produced by several variants of *M. tuberculosis*. The substantial protection afforded even in chronic situation throws evidence towards the potent immunogenicity of *M. habana*. Varying virulence of *M. tuberculosis* strains tested did not interfere with the protective efficacy of *M. habana*. Hank *et al*[7] reported a similar finding with BCG. *M. habana* has already been found to be non-pathogenic in several species of animals including monkeys[8]. *M. habana* has been found to elicit strong cell-mediated immune responses *in vitro* and *in vivo* against *M. tuberculosis* and *M. leprae* antigens indicating antigenic relationship[9]. The wide spectrum of immunity together with non-pathogenicity afforded by *M. habana* makes it a suitable candidate for future vaccine preparations. Further work is in progress.

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INHERITANCE OF INDUCED MUTANT CHARACTERS IN JUTE (*CORCHORUS CAPSULARIS* L.)

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THE dearth of distinct morphological characters in the natural variability seems to be responsible for the few genetic studies in jute (*Corchorus capsularis* and *C. olitorius*). Although the inheritance of some induced mutants was reported in the sixties[1,2], the data on the genetics of morphological traits are scanty, because the number of true breeding mutants available has been very small. Thus, in the case of *C. capsularis*, inheritance of only 19 mutants has so far been studied[3]. However, a very wide spectrum of mutants (111 mutants in *C. capsularis* and 136 in *C. olitorius*) and trisomic series have been established recently[4-7]. Using these, chromosome mapping and assigning of genes to different linkage groups should be possible. Towards this ultimate goal, the inheritance of 20 induced mutants of *C. capsularis* var. JRC 412 is reported in this paper, and the gene symbols have been proposed.

Eleven mutants were crossed reciprocally to the parent variety JRC 412 (referred as *P* in tables). For nine mutants, only one way crosses could be made. The F_2 population consisted of 2 to 10 F_1 plant