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Abstract

Typhoid is one of the most serious infectious bacterial diseases in third world countries. It is usually treated by traditional antibiotics but due to the appearance of antibiotic resistant strains physicians opt for phyto products and other alternative medicines for the treatment of typhoid. Baptisia, an extract from indigo plant root, has been proved to be highly effective ultradilute medicine for the treatment of typhoid; however, the mode of action of the ultradilute extract is uncertain. Due to the antigenic variations of Salmonella it seems to induce immuno system by activating both T and B cells by the formation of antibodies. This principle seems to be highly effective for the development of typhoid vaccine. The present studies found that Baptisia administration possibly caused a salmonella-like reaction in the body as this extract produces an endogenous antibody similar to salmonella reaction. Thus, this study suggests that Baptisia tinctoria extract can be used for the prevention and treatment of typhoid.

KEYWORDS: Antibody formation, typhoid, salmonella, ultradilute medicine, baptisia, bacterial antigens

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Introduction:

Typhoid is one of the most serious infectious bacterial diseases in third world countries. The global annual incidence is about 17 million cases, with approximately 600,000 deaths (Ivanoff, 1998; Pang *et al,* 1998). It is caused by the ingestion of food or water, contaminated with the Gram-negative short bacillus type bacterium *S. enterica* serovar Typhi (Giannella, 1996). Due to the presence of a special virulence factor and a capsule protein (virulence antigen) it can cause serious illness.

The cell wall of the bacteria contains a number of proteins and lipopolysaccharides that vary on the basis of molecular structures which are called antigens. *Salmonella* shows three types of antigens; 'O' or somatic / cell wall (lipopolysaccharides) antigens, 'Vi' antigen (it occurs in only three *Salmonella* serovars - Typhi, Paratyphi C, and Dublin) and 'H' or flagellar (flagellin) (Curd *et al*, 1998; Nalbantsoy *et al*, 2010). 'O' antigens are the polysaccharides associated with the outer leaflet of the outer cell membrane and 'H' antigens are the proteins associated with the flagella. On the basis of serotyping, a single species can be divided into different serotypes. All of the *Salmonella* serovars belong to two species: *S. bongori* containing eight serovars and *S. enterica* containing the remaining 2300 serovars which are divided into six subspecies - *S. e. enterica, S. e. salamae, S. e. arizonae, S. e. diarizonae, S. e. houtenae* and *S. e. indica* (Murray *et al,* 1995). The infectious dose of *Salmonella* is typically ~10^6 for a healthy adult but may fall very significantly if the stomach pH is increased. In such cases, the infectious dose appears to lie between 10 and 100 bacteria. After 6-8 days of infection, specific agglutinins (antigens) appear in the serum of the patient. A patient suffering from typhoid develops antibodies specific to the infecting organisms. To overcome this problem *S. enterica* can exhibit genetic switch so that the expression of 'H' antigen can be changed.

Although typhoid has been known for over two centuries and the causative agent was discovered in 1884, the disease has recently drawn the attention of medical practitioners as well as microbiologists due to the appearance of antibiotic resistant strains. During the last decade, antibiotic resistance and multi resistance of *Salmonella* sp. have increased to such an extent that it is challenging the arena of pharmacology. The major cause appears to be indiscriminate use of antibiotics in the treatment of bacterial diseases. Resistance to antibiotics against ampicillin, streptomycin, kanamycin, chloramphenicol etc in *Salmonella* strains involved in pediatric epidemics actually occurs due to mutation in the plasmid borne nucleic acids. To overcome this problem, phytoproducts are used for the treatment of typhoid (Singh *et al,* 2003; Arora *et al,* 2004; Sasikumar *et al* 2005; Owais *et al,* 2005).

The use of ultradilute medicines in the treatment of almost all types of diseases are widely known and the most dreadful life threatening disease - cancer is widely treated successfully by this system of medicine following The *Banerji Protocols* developed at the PB Homeopathic Research Foundation (PBHRF) in Kolkata (Pathak *et al*, 2003; Banerji *et al,* 2008; Frenkel *et al,* 2010). Baptisia along with several other medicines are used for the treatment of typhoid as per different symptoms. The use of Baptisia in typhoid fever and its efficiency has been verified by us, and at the clinics of the PBHRF, since more than three decades. Large numbers of patients treated by this medicine and the efficiency with which it works has compelled us to make this the drug of first choice in the *Banerji Protocols* for typhoid fever.

We site below, two anecdotal cases, from data of over a thousand, treated successfully with this medicine.

Case No. 1

IB, female, aged about 6 years, was suffering from pyrexia of seven days duration, when she was brought to our clinic. Her parents reported that the child had already been administered two courses of antibiotics, which had not made any impression on the fever. A thick white coated tongue, diffuse lower abdominal tenderness and minor hepatosplenomegaly prompted us to order a number of tests including the Widal antigen. This test showed a strongly positive reaction even at titers of 1:640. At that point, the child's temperature peaked at 104° F and we started the medicine Baptisia Mother Tincture, one dose = 5drops in one tablespoon of water, every three hours and some homeopathic analgesics to keep the temperature under control. The temperature responded by gradual regression and became normal i.e. 98º F, within three days. The Baptisia was continued, two doses daily, for another seven days to prevent a recurrence.

Case No. 2

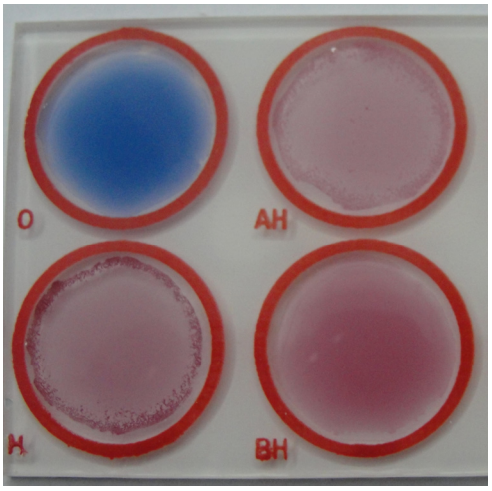
LKM, male, aged about 46 years, had already been diagnosed to be having typhoid, by a Widal test showing titers of 1:320. The patient was brought to us on the twelfth day of pyrexia in a debilitated state with a temperature of 104º F, coated tongue, delirium, rash on the chest and abdomen, a gastroenteritis with tenderness of the right lower abdomen and borborygmus. Management of the dehydration by the administration of oral rehydration therapy along with Baptisia Mother Tincture, one dose = 5 drops in one tablespoon of water, one dose every three hours, worked with remarkable effect. The symptoms were quickly brought under control within twenty four hours and the fever subsided within three days.

However, thorough study unveiling the microbiological process of its medicinal property is not yet explored. With a view to this objective, the present investigation was undertaken to explore the efficacy and mode of action of Baptisia in the treatment of typhoid as a solo medicine and in combination with others.

Materials and Methods:

Widal Antigen Reaction: The Widal, is the standard test for *Salmonella* infection. This is done to test for the presence of *Salmonella* antibodies, in significant concentration, in the blood of the patient. *Salmonella* exhibits somatic or cell wall (O) antigens and flagellar (H) antigens. These are detected by agglutination process with commercially produced antisera; the O antigens using a suspension of growth from an agar plate while the H antigens using a suspension of broth culture. The virulence of the bacteria was proved by the agglutination reaction using Widal antigens. A picture of the Widal slide showing agglutination with the Baptisia extract at 1:640 titre is presented as Figure 1.

Figure 1: A picture of the Widal slide showing agglutination with the Baptisia extract at 1:640 titre.

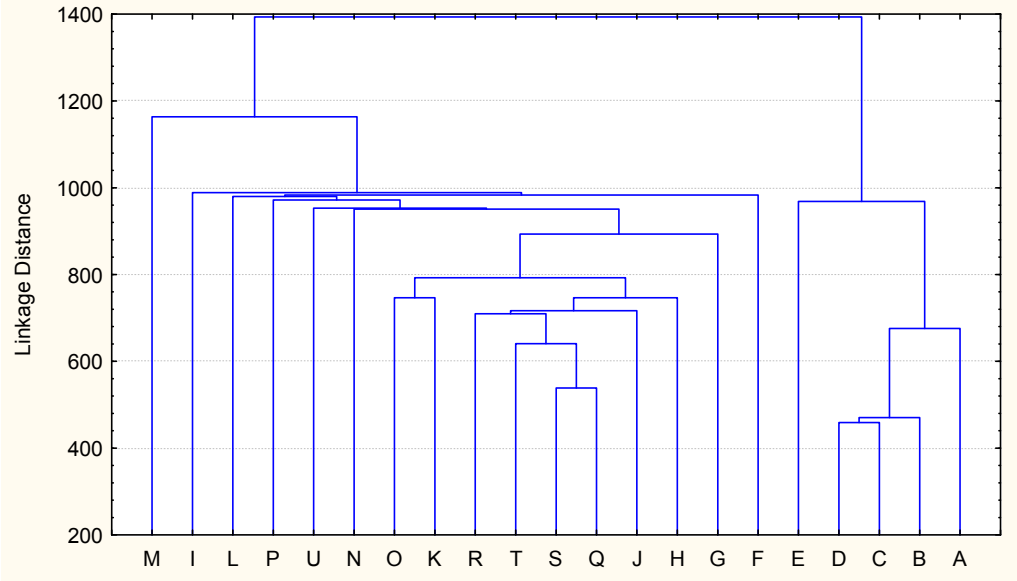


Statistical analyses:

The data was analyzed using ANOVA for two way classification. A principal component analysis was carried out to find clusters of antigens based on the titer values at which they agglutinate with plant extract. A multidimensional scaling (MDS) plot was obtained in this analysis which shows the cluster of antigens having almost similar agglutination pattern (see Figure 2). Further based on the

data of titer values of agglutination reaction, the effect of plant extracts appeared similar. Pair wise comparisons were carried out using paired t-test, which showed that within a group any two plant extracts did not differ significantly from each other for their action on different strains of the bacteria, but they do differ significantly with those forming different clusters.

Figure 2: Tree Diagram for 21 Variables Single Linkage Euclidean distances



Results:

Reactions of 66 different ultradilute medicines / plant extracts, with different strains of *Salmonella enteritis* as well as many diseased tissue extracts are shown in Tables 1 and 2 [ranging from 0 – 640 with 'no records' also], where, all zeroes / dots / blanks / dashes (-) are considered as zero, i.e. no reaction occurred. Each of these 66 ultradilute medicines has been matched with 13 disease conditions forming a matrix of 66 x 13 reaction results. This is taken as variable 1. The reaction matrix i.e. 1 x 13, 2 x 13, 3 x 13, and so on, for 66 extracts is taken as variable 2.

The two ways ANOVA examined the association between ultradilute medicines / plant extracts and disease conditions using different values. For example, item – 1 (Acon) of variable 1 may show significantly different pattern of reaction with disease conditions from item – 4 but may show closely similar, non significant different pattern of reaction with item – 10 or 11 and so on. Following this, groups / clusters of say item – 1, 10, 11 etc. of similar nature but not with those which shows significant different pattern of reaction with each other. Hence several clusters / groups of items (plant extracts) have been formed which give

similar results for particular disease conditions using different titer values of plant extracts but show difference between groups (Table 3).

Table 1: Reactions of different ultradilute medicines/ plant extracts for treatment of *Typhoid*

	To	Th	Ah	Bh	51	52	53	54	55	56	57	BT2	BT1
1. Acon	80	20	20	0	320	320	320	320	640	320	640	320	320
2. Ac phos	20	320	320	160	0	0	0	0	0	0	0	40	20
3. Adoni	40	20	0	0	0	20	0	0	160	0	0	40	160
4. Apocy	320	160	320	80	320	320	320	320	320	640	640	320	320
5. Aralia	0	0	0	0	0	20	0	0	160	20	20	20	320
6. Arjun	80	40	80	40	160	160	160	160	640	640	640	320	320
7. Aspid	20	0	0	0	80	20	40	40	320	320	320	40	160
8. Avena	0	0	0	0	0	0	20	20	20	80	40	40	20
9. Azadir	160	40	160	40	80	80	160	320	320	320	80	160	320
10. Bapt	0	0	0	0	160	640	640	640	640	640	640	320	640
11. BGBerb	80	0	0	0	160	320	640	320	320	320	320	40	360
12. Blatta	0	0	0	0	320	160	40	160	160	320	320	320	640
13. Brahm	0	0	0	0	0	160	40	40	80	320	80	20	160
14. Cactus	0	0	0	0	40	40	20	160	80	160	80	20	160
15. Calen	0	0	0	0	0	0	0	20	20	80	40	40	80
16. Canth	20	0	0	0	80	160	160	80	320	320	160	80	320
17. Caps	0	0	0	0	20	40	40	40	40	80	40	320	320
18. Cardm	0	0	0	0	0	0	0	0	0	0	40	80	160
19. Ceano	320	640	640	640	160	640	640	640	640	640	640	320	320
20. Cephi	0	0	0	0	20	20	0	20	0	20	20	0	40
21. Chelid	80	20	20	20	320	320	320	320	320	320	160	160	320
22. Chima	20	40	80	20	640	640	640	640	640	640	640	320	640
23. China	160	320	320	160	320	160	320	640	640	640	640	640	640
24. Chiona	160	40	160	20	80	160	160	40	160	320	320	320	320
25. Cina	20	0	0	0	20	40	0	20	40	20	0	0	40
26. Crata	40	0	0	0	80	80	80	40	320	320	160	80	320
27. Echina	0	0	0	0	0	0	0	0	0	0	0	20	320
28. Equis	0	0	0	0	0	0	0	0	0	20	0	0	80
29. Eucal	20	20	40	0	80	320	320	640	320	640	640	640	640
30. Eupat	20	0	0	0	20	40	20	0	20	160	0	40	80
31. Fucus	0	0	0	0	0	0	0	0	0	0	0	80	20
32. Geran	320	640	640	640	320	640	640	160	640	320	640	320	320
33. Ham	320	640	640	640	320	640	640	320	640	160	640	320	320
34. Hydra	40	0	0	0	320	320	320	320	320	320	640	320	320
35. J Ashok	160	320	80	80	160	640	160	320	320	640	640	320	640
36. Justiad	20	0	0	0	0	80	40	20	20	160	80	160	160
37. Kreos	20	80	20	160	20	0	0	0					
38. Kurchi	640	640	640	640	640	320	640	640	320	640	640	320	320
39. Mille	0	0	0	0	0	0	0	0	0	0	0	80	160
40. Myric	320	640	320	80	640	640	640	640	640	640	640	640	640
41. Pareibra	40	0	20	0	20	320	160	40	40	20	320	80	320
42. Passi	0	0	0	0	20	40	20	20	20	0	0	160	320
43. Piantb	0	0	0	0	0	20	0	0	0	0	0	160	320
44. Rauwo	40	0	20	0	160	640	640	320	320	640	640	320	640
45. Sabal	40	20	40	20	40	320	320	320	320	320	320	160	320
46. Stram	20	0	0	0	160	640	160	320	320	320	320	160	320
47. Symph	0	0	0	0	0	0	40	80	320	80	160	160	320
48. Syzy	160	160	160	160	160	640	320	320	640	320	320	320	640
49. TBP	0	0	0	0	20	40	40	0	20	0	0	80	160
50. TH	40	20	20	0	40	320	640	640	640	640	640	640	640

Table 2: Reactions of different ultradilute medicines / plant extracts for treatment of *Typhoid* (Continued)

	To	Th	Ah	Bh	51	52	53	54	55	56	57	BT2	BT1
51. Urtic	0	0	0	0	0	20	0	0	0	0	0	40	40
52. BO	0	0	0	0	0	20	0	0	0	20	0	160	160
53. Viton	0	0	0	0	20	40	20	40	20	0	20	320	320
54. Bap FM	640	640	640	640	640	640	640	640	640	640	640	640	640
55. Bapt SJ	40	40	80	40	40	640	640	640	640	640	640	640	640
56. Bapt 30	0	0	0	0	0	0	0	0	0	0	0	20	0
57. Bap200	0	0	0	0	0	0	0	0	0	20	0	20	0
58. Bapt 3x	0	0	0	0	0	0	0	0	0	0	0	0	0
59. China					640	640	640	640	640	640	640	640	640
60. Ashoka					40	320	640	640	640	640	640	320	320
61. Syzy					320	160	320	320	320	640	320	320	640
62. Ceanu					320	320	640	640	640	640	640	320	320
63. Chiona					160	160	320	160	80	80	320	320	320
64. Myric					640	640	640	640	640	640	640	640	640
65. Hamam					160	320	640	640	640	640	640	640	640
66. Bapt												640	

Table 3: Grouping of 66 different types of ultradilute medicines / plant extracts on the basis of agglutination reaction.

Group11	Group10	Group9	Group8	Group7	Group6	Group5	Group4	Group3	Group2	Group1
66 (Bapt)	30 (Iupat)	24 (Chiona)	18 (Cardm)	19 (Ceano)	7 (Aspid)	4 (Apocy)	5 (Aralia)	10 (Bapt)	1 (Acon)	2 (Ac Phos)
64 (Myric)	47 (Symph)	63 (Chiona)	51 (Urtic)	40 (Myric)	9 (Azadir)	6 (Arjun)	14 (Cactus)	22 (Chima)	11 (Berb)	3 (Adoni)
				54 (Bap FM)	16 (Canth)	35 (J Ashok)	43 (Piantb)	23 (China)	12 (Blatta)	8 (Avena)
				59 (China)	26 (Crata)	44 (Rauwo)	53 (Viton)	29 (Ucal)	21 (Chelid)	13 (Brahm)
				65 (Hamam)	41 (Pareibra)	48 (Syzy)	56 (Bapt 30)	32 (Geran)	34 (Hydra)	15 (Calen)
						61 (Syzy)	57 (Bapt 200)	33 (Ham)	45 (Sabal)	17 (Caps)
							58 (Bapt 3X)	38 (Kurchi)	46 (Stram)	20 (Cephi)
								50 (TH)		25 (Cina)
								55 (Bapt SJ)		27 (Echina)
								60 (Ashoka)		28 (Equis)
								62 (Ceanu)		31 (Fucus)
										36 (Geran)
										37 (Kreos)
										39 (Mille)
										42 (Passi)
										49 (TBP)
										52 (BO)

Discussion:

Salmonella infections are zoonotic and are due to ingestion of contaminated food. Currently, there are two recognized species: *S. enterica* and *S. bongori*, with six main subspecies: enterica, salamae, arizonae, diarizonae, houtenae, and indica (Janda and Abbott, 2006). A distinction is made between *Salmonella enteritis* and typhoid / paratyphoid *Salmonella,* where the latter *Salmonella enterica enterica* serovar Typhi because of a special virulence factor and a capsule protein (virulence antigen) can cause serious illness. Use of various phytoproducts against typhoid is widely accepted. The latex protein of *Calotropis procera* has been proved highly effective against *Salmonella* (Lima-Filho *et al*, 2010).

Salmonella antibodies were first found among Malawi children (MacLennan *et al,* 2008). The antibody protects children against *Salmonella* infections. A study of 352 children at Blantyre's Queen Elizabeth hospital found that children up to two years old develop antibodies that aid in killing the bacteria. The researchers proposed that this could lead to a possible *Salmonella* vaccine (Panthel *et al,* 2008; Xu *et al,* 2010).

Following *Banerji Protocols*, in our daily practice, ultradilute form of Baptisia singly or in combination with other medicines (Table - 2) yields most satisfactory results against typhoid. Agglutination reaction seems to involve molecular alteration that occurs between antigenic epitopes and medicinal as well as chemical constituents. It seems to corroborate the findings of contemporary investigators regarding the effects of *Salmonella* infection at the molecular level. It clearly approves direct participation of the immune system to combat the bacterial infection. *Salmonella* antigens seem to induce the generation of specific $CD4^+$ and $CD8^+$ T cells and both T cell populations are important for protection during primary and secondary responses, although the mechanisms underlying T cell-mediated protection are not yet completely understood. Treatment of *Salmonella* with anti-Salmonella IgG enhanced the macrophages' uptake of bacteria and induced high frequency apoptotic cell death. Humoral immunity cooperates with cell-mediated immunity upon induction of apoptosis in host cells to establish protective immunity against *Salmonella* infection (Eguchi and Kikuchi, 2010). *Salmonella* antigens are also reported to induce antibody production against the agglutinins of Bacteria, thus it raises the natural bodily resistance to the invasion of the bacillary intoxication, which produces the typhoid syndrome. The immune reaction involves both T and B cell-mediated responses (Mittrucker and Kaufmann, 2000). Karasova *et al* (2010) showed that *Salmonella* infection led to the depletion NK cells. It induces receptors on monocytes, macrophages and dendritic cells that activate lymphocytes to produce interferon (IFN)-γ, Interleukin (IL)-12 and IL-23 initiating a cytokine immune response (van de Wetering *et al*, 2009).

Antigenic profiles and agglutination reactions led the scientists to think one step ahead of the mere administration of ultradilute medicines. Scientists around the world (Panthel *et al*, 2008; Xu and Hensel, 2010; Xu *et al*, 2010) have already done a lot of molecular investigations regarding development of typhoid vaccine. Three types of typhoid vaccines are currently available; an oral live-attenuated vaccine; a parenteral heat-phenol-inactivated vaccine and a newly licensed capsular polysaccharide vaccine for parenteral use. Typhoid vaccine is strongly recommended to travelers to parts of the world where typhoid is endemic, people who remain in close contact with typhoid carriers and laboratory workers who work with *Salmonella* Typhi bacteria. Our investigation which mainly involves *in vitro* agglutination reaction and administration of Baptisia and other ultradilute medicines undoubtedly explains the molecular mechanism of vaccine development. Inoculation of live or dead antigenic fragments of *Salmonella*, no doubt induces the immune system to develop antibodies.

Thus this investigation confirms Baptisia, singly or in combination with other medicines is a potent anti typhoid ultradilute medicine and its mode of action is to mimic the *Salmonella* immune reaction that causes diseases like typhoid by producing endogenous antibodies without having an actual *Salmonella* infection. Thus this novel finding suggests that Baptisia can be used for the prevention as well as the cure of the disease. Further study in this respect may help to develop potent vaccines against typhoid.

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