

**IN VITRO CLUMPING OF FOOD POISONING STRAINS OF SALMONELLA BY
EXTRACT OF *BAPTISIA TINCTORIA* (*BAPTISIA* Θ)**

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ABSTRACT

Baptisia tinctoria extract is a well known medicine which is used for the treatment of typhoid fever in ultra diluted form in homeopathic practice under alternative medicine. However, there is no study about its direct action on *Salmonella*. In the study, we made experiments to see whether *Baptisia tinctoria* extract as a homeopathic medicine prepared according to Government approved Homeopathic Pharmacopoeia (*Baptisia* Θ), 10g per dL can react with food poisoning strains of genus *Salmonella*. In this experiment, we have used four food poisoning strains of *Salmonella* namely *S. Enteritidis* (antigenic formula I 9,12:g,m:-), *S. Orion* (antigenic formula I 3,15:y:1,5), *S. Istanbul* (antigenic formula I 8:z10:e,n,x), *S. Kentucky* (antigenic formula I 8:i:z6); a saline suspension of each bacteria was challenged with the *Baptisia* extract *in vitro*. It was found that *Baptisia* can induce a prominent clumping reaction in all the tested *Salmonella* strains. Similarly another control experiment was done with *Ipecacuanha* extract (*Ipecacuanha* Θ) and *Arsenicum album* instead of *Baptisia* in the experiment, which showed no clumping with *Salmonella* spp. The exact mechanism is still unknown, but it appears to be due to specific arabinogalactan proteins of the plant creating an antigen antibody mimicry with the bacteria which may be responsible for this reactions.

KEYWORDS: *Baptisia*, Non typhoidal *Salmonella*, Clumping reaction.

INTRODUCTION

Baptisia tinctoria (L.) R.Br (Fig.1) is a perennial herb belonging to Leguminosae family, commonly known as wild indigo growing in many parts of United States. It is a plant of about 1.5m height. Its leaves are palmately compound and is made up of three leaflets radiating from one point which are cuneate to obovate, about 1.5cm long. Flowers are bright yellow in color of about 1.5cm length with racemose type of inflorescence. Roots are short, blackish, woody in texture.



Fig.1 : Diagram of *Baptisia tinctoria*

Salmonella spp. are gram negative, rod shaped, motile bacteria with peritrichous flagella, belonging to the Enterobacteriaceae family. It is about 1-3x0.5 μm in size. Its cell wall contains various antigens which vary on the basis of their molecular structure namely (i) Flagellar antigen H, (ii) Somatic antigen O and (iii) Surface antigen Vi, depending on which they are classified into various serological groups. The genus *Salmonella* is basically divided into two major species, *Salmonella enterica* and *Salmonella bongori*. Again the enterica species is classified into various sub species i.e, *S. salamae*, *S. arizonae*, *S. diarizonae*, *S. houtenae* and *S. indica*. The serotype *S. Typhi*, *S. Paratyphi A* and *S. Paratyphi B* are important pathogens to human beings producing various clinical syndromes like enteric fever, gastroenteritis or food poisoning and septicemia with or without local suppurative lesions. There are also many important food poisoning species of *Salmonella* which are about of 70 strains, among them serotype Typhimurium, *S. Enteritidis*, *S. Agona*, *S. Newport*, *S. Heidelberg*, *S. Poona*, *S. Bereilly* are well known as they can cause infection easily in general population.

Baptisia tinctoria Θ has an evident action in the treatment of typhoid fever. *Baptisia* when introduced to

human body in low potency produces a form of antibodies called agglutinins which are normally not found in human blood and it reacts with typhoid bacilli to form clumps. Thus helping defense system against the disease.

Homeopathy is a system of alternative medicine promulgated by Dr. Christian Friedrich Samuel Hahnemann, a German physician in the year 1796. It is based on definite law of 'Similia Similibus Curantur', which means like cures like. In this system of medicine selection of remedy is based on the principle that it has the capability of producing similar symptoms of the disease to be cured in the healthy person. The medicine acts by stimulating the immune system of the individual, so that the body can arouse itself to fight against the disease. The homeopathic medicines are used in ultra diluted form, so as to avoid unnecessary aggravations that can take place if it is used in its crude form. Though the exact mechanism of action of the homeopathic medicines is still unknown but it proves out to be effective in clinical approach.

MATERIALS AND METHODS

The bacterial strains

In this experiment we have used four non typhoidal *Salmonella* strains, *S. Enteritidis* (antigenic formula I 9,12:g,m:-), *S. Orion* (antigenic formula I 3,15:y:1,5), *S. Istanbul* (antigenic formula I 8:z10:e,n,x), *S. Kentucky* (antigenic formula I 8:i:z6), which were obtained from World Health Organisation External Quality Assurance Program on *Salmonella*. All the strains are maintained as stock cultures, and before the main experiment fresh sub cultures were finally made on nutrient agar medium and the sub cultures were used in this experiment.

Baptisia tinctoria extract, *Ipecacuanha* extract and *Arsenicum album* preparation

These are readily available as homeopathic medicines which were procured from a reputed company (HAPCO, Kolkata, India), these medicines are prepared strictly following Homeopathic Pharmacopoeia as per guidelines given by Government of India. In this experiment only crude extract (1:10 dilution, known as mother tincture in homeopathy) was used.

The Experiment

Uniform suspension of all the bacteria were prepared on clean, grease free glass slides with 50µL of normal saline in biosafety cabinets. After this 50µL of each extract or preparation was thoroughly mixed with the suspensions; then the slides were gently tilted for 2 minutes, and then examined under low power (10X objective) of the microscope to see if clumping was present. A semi quantitative estimation of the clumping was done as 1+, 2+, 3+ and 4+, depending on the relative size of the clumps, and rapidity of their formation. Control experiments with *Ipecacuanha* Θ and *Arsenicum album* Θ were similarly done.

RESULTS

All the four strains of non typhoidal *Salmonella* -S. *Enteritidis* (antigenic formula I 9,12:g,m:-), *S. Orion* (antigenic formula I 3,15:y:1,5), *S. Istanbul* (antigenic formula I 8:z10:e,n,x), *S. Kentucky* (antigenic formula I 8:i:z6) showed 4+ clumping reaction with *Baptisia* Θ (Table 1, Fig. 2). The experiments with *Ipecacuanha* Θ and *Arsenicum album* Θ instead of *Baptisia* Θ showed no clumping reaction in general, except two strains of *Salmonella* which showed faint clumping reactions, with *Ipecacuanha* Θ (Table 1, Fig. 2).

Table1: Clumping reactions of Salmonella strains with Baptisia extract and controls.

	<i>Baptisia tinctoria</i> Θ	<i>Ipecacuanha</i> Θ	<i>Arsenicum album</i> Θ
<i>S. Orion</i>	Clumping(4+)	Clumping (\pm)	No Clumping
<i>S. Istanbul</i>	Clumping (4+)	No Clumping	No Clumping
<i>S. Enteritidis</i>	Clumping (4+)	No Clumping	No Clumping
<i>S. Kentucky</i>	Clumping (4+)	Clumping (\pm)	No Clumping

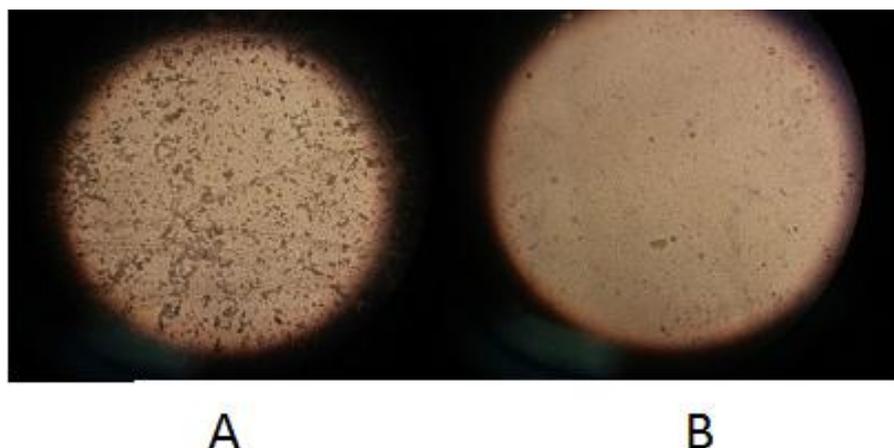


Fig.2: Clumping reaction with Baptisia (A), no reaction with Ipecacuanha (B)

DISCUSSION

There are only a few studies with *B. tinctoria*. Almost all these studies were oriented to the composition particularly in relation to glycosides (Wagner et al, 1969), and stimulation of immunity (Beuscher and Kopanski, 1985). The constituents of *B. tinctoria* are alkaloids, glycosides and oleoresin, and it also contains isoflavones which are oestrogenic, flavonoids and polysaccharides. In 1992, Egert and Beuscher studied antigen specificity of immune reactive arabinogalactan proteins from this plant (Egert and Beuscher, 1992). They performed series of experiments on cross reactivity of antibodies which were raised against arabinogalactan proteins derived from *B.tinctoria*. They performed competitive ELISA test to prove specific glycoproteins of *B. tinctoria* which were not found in other tested plants. It was also found that lipopolysaccharides from *Salmonella Typhimurium* can inhibit such antigen – antibody reaction. More recently Classen *et al* (2006) studied immunomodulatory effects of arabinogalactan proteins from *B. tinctoria* and they observed that there was proliferation and increased IgM production by lymphocytes under influence of arabinogalactan proteins of *B. tinctoria*. Thus this clumping reaction may be an agglutination reaction with specific arabinogalactan of *B. tinctoria* which is absent in other plants.

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REFERENCES

1. Beuscher N, Kopanski L. Stimulation of immunity by the contents of *Baptisia tinctoria*, *Planta Med.*, 1985; 5: 381-4.
2. Classes B, Thude S, Blaschek W, Wack M, Bodinet C. Immunomodulatory effects of arabinogalactan proteins from *Baptisia* and *Echinacea*, *Phytomedicine*, 2006; 13(9-10): 688-94.
3. Egert D, Beuscher N. Studies on antigen specificity of immunoreactive arabinogalactan proteins extracted from *Baptisia tinctoria* and *Echinacea purpurea*, *Planta Med.*, 1992; 58(2): 163-5.
4. Wagner H, Horhammer L, Budweg W, Major A, Farkas L. Investigation of the glycosides from *Baptisia tinctoria*. 3. Synthesis of pseudobaptisins, *Chem Ber*, 1969; 102(9): 3006-8.