

EVALUATING THE EFFECTS OF HOMOEOPATHIC DILUTION ATROPA BELLADONNA ON STREPTOCOCCUS PYOGENES: AN IN-VITRO STUDY

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ABSTRACT

Background: *Streptococci* are gram positive cocci arranged in chains. They are important human pathogens, causing pathogenic infections with a characteristic tendency to spread. *Streptococcus Pyogenes* colonizes in the human upper respiratory tract- throat, nasopharynx and nose of patients and carriers. Symptomless infection is common and helps to maintain the organism in the community.

Material and methods: Samples procured from MTCC was incubated to grow a mother culture of *Streptococcus Pyogenes*. Gram staining confirmatory test was performed to confirm the obtained grown organism was *Streptococcus Pyogenes*. Further subcultures were made from the mother culture in which disc method was used to assess the In-Vitro antibacterial effect of potentised homoeopathic drugs against human pathogen *Streptococcus Pyogenes* and compared with action of standard antibacterial drugs penicillin (control), amoxicillin (control) and rectified spirit (control/vehicle) by "Inhibition zone technique".

Results: Homoeopathic potentized drug dilution *Atropa belladonna* in respective potencies were tested against *Streptococcus Pyogenes* in which *Belladonna 12C* showed maximum zone of inhibition of 5mm.

Conclusion: The results of this experiment support the concept of "evidence-based medicine" depict that homoeopathic medicines not only work in in-vivo but are equally effective in in-vitro conditions having definite inhibitory activity against *Streptococcus Pyogenes*.

INTRODUCTION

In the present scenario, incidences of emerging/re-emerging infectious diseases affect human health despite extraordinary progress in the area of biomedical knowledge. Regions of world with low income and poor infrastructure suffer a high burden of “*Streptococcus Pyogenes*” – A gram positive cocci arranged in chains, causing pyogenic infections. They colonize in the upper respiratory tract, carrier rates of up to 20% have been observed which are more frequent in children at 5-8 years of age, though they affect adults and children of other age groups. They are common in winter in temperate countries. The bacteria are responsible for causing suppurative respiratory diseases. Several antibiotics have been developed against the bacteria and have been in use for a long time in allopathic system of medicine. But the bacteria appear to have developed drug resistance in due course of evolution. Homoeopathy offers a beneficial role in treating infectious diseases caused by pathogenic microorganisms. In recent years many homoeopathic drugs have shown to possess anti-bacterial properties in clinical trials. This study is an effort to witness the effect of homoeopathic drugs in higher dilutions on human pathogenic microorganism *Streptococcus Pyogenes* In-vitro.

Objectives: To culture human pathogenic bacteria *Streptococcus Pyogenes* and to study and witness the action of potentized homoeopathic drug *Atropa belladonna* in higher dilutions on pathogenic microorganism *Streptococcus Pyogenes*.

LITERATURE REVIEW

Streptococci are gram positive cocci arranged in chains. They are important human pathogens, causing pathogenic infections with a characteristic tendency to spread. Rosenbach (1884) isolated the cocci from human suppurative lesions and gave them the name “*Streptococcus Pyogenes*”

Streptococcus Pyogenes colonizes in the human upper respiratory tract- throat, nasopharynx and nose of patients and carriers. Symptomless infection is common and helps to maintain the organism in the community. The major source of *Streptococcus Pyogenes* is the human upper respiratory tract. A streptococcal infection of the respiratory tract is more frequent in children 5-8 years of age. It is common in winter in temperate countries. No seasonal distribution has been identified in tropics.

Streptococcus Pyogenes is responsible for causing inflammatory and suppurative diseases.

Immunity is type specific and appears to be associated with the antibody to the M protein. Re-infections occur because of the multiplicity of serotypes. Infection is transmitted by Direct contact, contaminated dust or fomites, Transmission through milk from cattle, Insects (eye gnat *Hippelates*)

Various structural components of *Streptococcus Pyogenes* exhibit antigenic cross reactions with different tissues of the human body. It has been postulated that these

antigenic cross-reactions may account for some manifestations of rheumatic fever and other streptococcal diseases, the tissue damage being an immunological factor.

Cell wall associated factors: Capsule inhibits phagocytosis – non-antigenic in humans
Carbohydrate antigens show cross reactivity in humans. Protein antigens are M proteins, T proteins, R proteins.

Human pathogen *Streptococcus Pyogenes* possess toxins such as Hemolysin that produce streptolysin 'o' and 's', Streptococcal pyrogenic exotoxin that produces erythematous reaction, Streptokinase promotes lysis of human fibrin clots, Deoxyribonucleases cause depolymerization of DNA, Hyaluronidase enzyme breaks down hyaluronic acid of tissues, Serum opacity factor is the virulence determinant of the organism.

The diseases caused by *Streptococcus Pyogenes* can be suppurative or non-suppurative, which include the sequelae to post-streptococcal infections. *Streptococcus Pyogenes* produces pyogenic infections with a tendency to spread locally, along lymphatics and through the bloodstream.

Inflammation:

Inflammation is recognized as a set of complex changing responses to tissue injury primarily caused by toxic chemicals, some environmental agents, trauma, overuse, or infection. Some of these responses can be beneficial in wound healing and infection control or pathological as in many chronic disease states. Acute inflammation has a rapid onset of minutes or hours, usually resolves in a few days, has classic signs and symptoms, and has cellular infiltrate primarily composed of neutrophils. Chronic inflammation has a slow onset of days, a long duration of years, less prominent classical signs and symptoms, and cellular infiltrate primarily composed of monocytes/macrophages and lymphocytes.

Suppurative diseases

Respiratory infections: The primary site of infection of the human body by *Streptococcus Pyogenes* is the throat. Sore throat is the most common streptococcal disease. It may be localized as tonsillitis or may involve the pharynx diffusely. From the throat, streptococci may spread to the surrounding tissue, leading to suppurative complications. Streptococcal pneumonia seldom follows throat infection but may occur as a complication of influenza or other respiratory viral diseases.

Skin and soft tissue infections: *Streptococcus Pyogenes* causes a variety of suppurative infections of the skin, including infections of wounds or burns, with a predilection to produce lymphangitis and cellulitis. Infection of minor abrasions may at times lead to fatal septicaemia. The two typical streptococcal infections of the skin are erysipelas and impetigo.

Erysipelas is a diffuse infection involving the superficial lymphatics.

Impetigo is caused by a limited number of serotypes of *Streptococcus Pyogenes*. It is one of the main causes of acute glomerulonephritis.

Necrotizing fasciitis: streptococcal subcutaneous infections range from cellulitis to necrotizing fasciitis. the latter condition is more commonly caused by a mixed aerobic and anaerobic bacterial infection, but some strains of *Streptococcus Pyogenes* particularly “M types 1 and 3 forming pyrogenic exotoxin A” may alone be responsible.

Toxic shock syndrome: soft tissue infections with some M types of *Streptococcus Pyogenes* may sometimes cause a toxic shock syndrome resembling Staphylococcal TSS.

Genital infections: *Streptococcus Pyogenes* was an important cause of death due to puerperal sepsis, with an infection being exogenous, in the pre-antibiotic era. ***Non-suppurative diseases***

- acute rheumatic fever
- post streptococcal glomerulonephritis

Atropa belladonna

Atropa belladonna is commonly known as the deadly nightshade is known for its action in treatment of acute inflammatory illness. *Heat, redness* and *burning* are three great characteristic notes of *Atropa belladonna* and are constantly cropping out in the pathogenesis. The face is purple, red, and hot, or yellow. Redness and pallor alternate. Its sphere of action covers all kinds of inflammation, congestion, fever and suppurative illness. Belladonna has been effective in symptomatic treatment which run parallel to streptococcal infections.

Research philosophy: *Streptococcus Pyogenes* are responsible for causing suppurative respiratory diseases. Several antibiotics have been developed against the bacteria and have been in use for a long time in allopathic systems of medicine. But the bacteria appear to have developed drug resistance in due course of evolution. Homoeopathy offers a beneficial role in treating infectious diseases caused by pathogenic microorganisms. In recent years many homoeopathic drugs have shown to possess anti-bacterial properties in clinical trials. This study is an effort to witness the effect of homoeopathic drugs in higher dilutions on human pathogenic microorganism - *Streptococcus Pyogenes*.

Research approach:

Sample of *Streptococcus Pyogenes* procured is to be grown in an enriched culture media to check for antibacterial properties where the cultures will be laid with discs soaked in dilutions of potentised homoeopathic drugs. The data will be collected based on the zone of inhibition that is to be measured and analysed. A confirmatory test will be performed on the mother culture and a control study (penicillin and amoxicillin antibiotic discs) and a vehicle study

will be performed with rectified spirit.

METHODOLOGY

The isolated dried freeze sample of *Streptococcus pyogenes* is to be diluted in an enrichment medium which should be stored under sterile premises. A gram staining test has to be performed as a confirmatory test for identification of the bacteria. Blood agar being a suitable culture media for the growth of streptococcus bacteria should be prepared and poured onto sterile petri dishes that are left to settle incubator at a suitable temperature for 24 hours to confirm the absence of any possible contamination. The diluted bacterial sample is inoculated by streak method in the blood agar culture plate which should be kept in incubator at 37 °C for 3 days. check for bacterial growth in the inoculated culture plate. If bacterial growth is observed in the streaked area, it confirms for a pure culture. Obtain sample from the streaked area of bacterial growth and inoculate completely in multiple blood agar culture plates to which sterile discs soaked with dilutions of potentised drug dilution of homoeopathic medicine *Atropa belladonna* and antibiotic discs for control study. Sterile discs soaked in rectified spirit are also placed in bacterial culture plates subjected for vehicle study.

OBSERVATION

Table : 1

Homoeopathic drugs	Zone of Inhibition
Belladonna 6C	No inhibition
Belladonna 12C	5mm
Belladonna 200C	1mm
Belladonna 1M	1mm
Control/vehicle study	Zone of Inhibition
Amoxicillin	1mm
penicillin	1.5mm
Rectified spirit	1mm

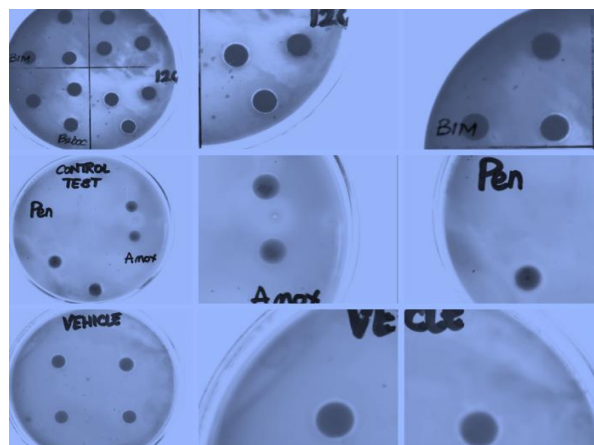


Figure1

RESULT

Homoeopathic potentized drug dilution *Atropa belladonna* in respective potencies were tested against *Streptococcus Pyogenes* in which Belladonna 12C showed maximum zone of inhibition up to 5mm as seen in figure 1.

DISCUSSION

The result obtained clearly explains the zone of inhibition of the human pathogenic organism *Streptococcus Pyogenes* in homoeopathic drug *Atropa belladonna* in higher dilutions. It has been widely believed that homoeopathic system of medicine is pseudoscience and is an alternative system of medicine as the highly potentised drugs were believed to not carry material drug substance and hence its efficiency in treatment has also been widely question to be a placebo effect.

Homoeopathic drugs are prepared by trituration and diluting the original drug substance. As modern science was at the opinion that substance does not exist beyond Avogadro limit of $6.02214076 \times 10^{23}$. With respect to this homoeopathic drug potencies beyond 12C were imposed to not have drug substance that they do to produce efficient results in treatment. This laid a strong need for effective laboratory study.

This effort to study the antimicrobial effect of homoeopathic potentised drug *Atropa belladonna* in-vitro has presented a zone of inhibition against human pathogenic bacteria *Streptococcus Pyogenes*. A

maximal zone of inhibition of 5mm is witnessed in *Atropa belladonna* 12C. Zone of inhibition noted in control study of penicillin was 1.5mm and amoxicillin was 1mm and vehicle study with rectified spirit had a zone formed of 1mm.

The in-vitro study states that higher dilutions of potentised homoeopathic drugs have antimicrobial properties against the human pathogenic bacteria *Streptococcus Pyogenes* and that the effect of potentised drugs will not be hindered by Avogadro limit. This is of great significance.

CONCLUSION

The results obtained in the in-vitro study reveals that homoeopathic drug *Atropa belladonna* has a marked inhibitory activity against *Streptococcus Pyogenes*. It contributes the concept that homoeopathy is an evidence-based medicine and **confirms the anti-inflammatory and anti-bacterial properties of *Atropa belladonna***. It is also fortifying the effect of homoeopathic drugs that zone of inhibition witnessed in *Atropa belladonna* 12C is greater than penicillin, amoxicillin and rectified spirit. Further studies may open new prospect in the treatment of human pathogenic bacteria.

These results will definitely clear the misconception that Homoeopathy is not a placebo therapy and that the effect of potentised drugs will not be hindered by Avogadro limit, it will have to be accepted as medicine of science.

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