Evaluation of the Efficacy and Safety of Potassium Aluminium Tetraoxosulphate (Vi) (ALUM) in the Treatment of Tuberculosis

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Abstract: Tuberculosis is the second leading cause of death worldwide killing 2 million people each year. In an effort to develop a new anti-tuberculosis agent that would be effective against both drug susceptible and drug resistant strains of Mycobacterium tuberculosis, an in vitro study on efficacy and safety of Potassium Aluminum Tetraoxosulphate (vi) (Alum) in the treatment of tuberculosis was carried out using the proportion method. The results showed that at the highest concentration of 0.003g/ml, Mycobacterium tuberculosis was resistant to the alum extract while the standard drug (streptomycin) inhibited the growth of Mycobacterium tuberculosis at the same concentration. The histological analysis of the various organs showed normal morphology and no inflammation was seen. Statistical analysis of the weight of the experimental animals compared with those of the controls showed no significant weight difference and no mortality was recorded throughout the experimental process. The histological studies suggest that alum was relatively safe for mammalian consumption at the concentration used, but was ineffective against Mycobacterium tuberculosis.

Key words: Missing

INTRODUCTION

Tuberculosis is a major cause of illness and death worldwide especially in Africa and Asia. In 2007, there were an estimated 9.27 million new cases of tuberculosis worldwide [1] and in Nigeria, a prevalence of 460,000 has been reported [2]. Due to the resistance of Mycobacterium tuberculosis to popular orthodox treatment, there is now a renewed interest in the use of natural products for chemotherapy. It has also been reported by many workers that a large proportion of Nigerian population depend heavily on natural products for cough treatment [3].

A number of experiments have been carried out on the antimicrobial effects of different extracts on cough pathogen [4]. Worked on the antimicrobial activity of crude extracts of twelve medicinal plants and “Epa-ijebu” (a “wonder cure” concoction) used in south western Nigeria on five common bacterial pathogens. His findings showed that extracts of Allium cepa, Xylopia aethiopica, Allium ascalonicum, Allium sativum, Aframomum melegueta and Terminalia glaucescens inhibited the growth of the test organisms.

There is therefore a need to systematically and scientifically evaluate these claims with a view to authenticating them in order to establish their viability.

Alum (the crystallized double sulphates with the formula KAl(SO4)2 12H2O are generally odourless, colourless crystalline solids that turn white in air [5]. Reported that one of the ingredients for the cure of cough was alum [6]. Stated that a suspension of alum precipitated diphtheria toxoid and had a much higher immunogenicity than the fluid toxoid. Even though a number of reports stated that alum-adjuvanted vaccines were no better than plain vaccines [7], the use of alum as an adjuvant is now well established.

According to [8], alum promotes a strong humoral response to diphtheria, tetanus and hepatitis B vaccines and is widely used in bacterial vaccines. Alum is also widely used in rural areas of Nigeria for the treatment of pediatric cough (personal communication).

With the increasing incidence of HIV/AIDS in Nigeria and the role of tuberculosis as the leading opportunistic infection in the disease, there is the increasing need to research into new and readily available, acceptable and efficacious treatment options for the generality of our...
populace. In this new focus area, natural products are the preferred options as available statistics have proven that majority of Nigerians especially in the rural areas still depend on them for the maintenance of their health [9].

MATERIALS AND METHODS

Safety Evaluation

Test Animal: Forty Swiss albino mice (all males) of age 16 weeks and weighing 21-25g were used to assess the safety of the test chemical (alum). The mice were allotted into four groups and allowed 2 weeks to acclimatize to the experimental conditions.

Test Chemical: The alum used in this project work was purchased from a local market in Lagos metropolis. It was authenticated in the department of chemistry, university of Lagos, as potassium aluminum tetraoxosulphate (VI).

Evaluation of the Efficacy of Drug (Alum) Mixture

Preparation of Culture Medium: Twelve freshly laid chicken eggs (not more than 24 hours old) were washed with detergents and sterilized with commercially prepared methylated spirit. The eggs were cracked aseptically into a sterile blender followed by the addition of 300mls of mineral salt solution and 100mls of 2% malachite green. The whole mixture was blended intermittently at 10 seconds interval and the resulting mixture was allowed to settle. 7-10mls of the mixture was dispensed into 14 sterile universal containers using a dispenser.

The alum was crushed into coarse powder by grinding in a clean mortar with pestle. Five grams of the powdered alum was dissolved in 150mls of distilled water and allowed to stand for 24hrs till it was finally dissolved. (This was done according to the recipe for cough treatment by the traditional health practitioners in rural Nigeria).

Preparation of Alum and Drug Containing Media:

Lowenstein - Jensen’s (LJ) medium was used for all susceptibility testing. The alum mixture was further diluted with sterile distilled water to concentrations of 0.0033g/ml, 0.00033g/ml and 0.000033g/ml respectively. The same concentrations were also used to prepare a standard drug medium (streptomycin), which served as the control medium for the experiment.

For each concentration of alum and streptomycin, two LJ slopes were prepared. 1ml of each concentration of alum and the standard drug were added to 9mls of freshly prepared Lowenstein - Jensen’s medium and inspisated at 85°C for 45 minutes. The sterility check was carried out by allowing the solid medium to stand at room temperature for 24hrs. Thereafter, the medium was stored in the refrigerator at 4°C, until ready for use.

Inoculation of LJ Slopes Containing Alum and the Standard Drug with Bacterial Culture: The proportion method described by Ait-khaled and Enarson, 2003 was used. 1mg of the organism (Mycobacterium tuberculosis) (MIC quantification) was introduced into MC - Cartney bottles, containing 2mls of distilled water together with 4 glass beads. The MC - Cartney bottles were shaken for 20-30seconds using a vortex mixer. 5mls of distilled water was added slowly while continuously mixing. The opacity of the bacterial dilution was adjusted by the addition of distilled water to get a standard suspension using the MaC - Farland No. 1 as a comparative standard.

1.0ml of the bacterial suspension was taken from the MC - Cartney bottles and discharged into 9mls of distilled water in a test tube to produce the first dilution of 10^-1mg/ml. In the same way, 1mg of the bacterial suspension was discharged into the next test tube containing 9mls of distilled water to produce the 2nd dilution of 10^-2mg/ml. Further serial dilutions were done until the 5-fold dilution steps were achieved.

The entire procedure discussed above, for the inoculation of the bacteria into the medium was repeated for four more patients, whose sputum samples were confirmed to produce pure isolates of Mycobacterium tuberculosis after the Ziehl - Nelson’s staining procedure, microscopy and biochemical tests.

The bacteria dilutions of 10^-3mg/ml and 10^-4mg/ml earlier discussed, were inoculated on each slopes with alum and the standard drug (control set up). They were labeled in pairs (which correspond to the double LJ slopes prepared initially for the different concentrations) as 10^-3mg/ml and 10^-4mg/ml respectively.

The inoculated slopes were loosely closed with a cap to allow for evaporation and then placed in the incubator at 37°C. The liquid part of the inoculums had evaporated after 24hrs, so the caps of the universal containers were firmly closed and left to incubate at 37°C for a period of 28 days.

Evaluation of Safety

Preparation of Drug (Alum) Mixture and Administration on Test Animals: Five grams of alum was weighed using a top loading weighing balance. It was dissolved in 750ml of distilled water (according to market specification) and left to stand for 24hrs at room temperature, until it was
fully dissolved. The volume of alum required for a mouse weighing 20.7g was calculated following the quantity said to be administered orally to a child weighing 10kg.

The average concentration for each mouse was rounded off to 0.01ml. The concentrations given to mice in the different cages varied, due to the experimental requirements, as shown below;

Group A animals were given 0.001ml of the alum mixture. 
Group B animals were given 0.01ml of the alum mixture. 
Group C animals were given 0.1ml of the alum mixture. 
Group D animals were set as control for the experiment.

The administration was done eight hourly for 4 days and the drug mixture was kept in the fridge after every drug administration, until the next series of administration.

Assessment of Mortality: Individual mouse was taken to be dead if the mouse does not show any form of movement, even when disturbed. The animal remains still and non-active.

Histological Studies: After the last day of administration, 4 mice from each cage were sacrificed by first, anesthetizing them with chloroform followed by dissection using surgical blades. The brain, lungs, liver and kidney were gently removed and fixed in 10% formalin for histological analysis. The same process of sacrificial killing was carried out 2 weeks later on three representatives of each group. The same process was repeated for the three representatives of each group left after 3 weeks and the same organs were removed for histological analysis.

The tissues were preserved and transported in a fixative; 10% neutral buffered formalin to prevent autolysis. The tissues were cut into smaller bits and then dehydrated in graded alcohol (50% alcohol for 1hour, then 70% alcohol for 6hours, 90% alcohol for 6hours, 95% alcohol for 1hour and 100% (I) alcohol for 2hours, 100% (II) alcohol for 2hours and 100% (III) alcohol for 1hour). The tissues were then cleared in xylene for 5hours (de-alcoholisation), before they were impregnated in 3 changes of paraffin wax, with melting point of 56.0°C, 1hour each. The sections of these various tissues were then embedded in fresh wax. Serial sections of 3ìm thickness were cut in rotary microtome and passed through xylene for 2 hours, followed by absolute alcohol and water to hydrate the sections. The sections were stained with haematoxylin and eosin, dehydrated in graded alcohol, cleared in more xylene to dewax completely and mounted in Canada balsam. The slides were left to dry on the hot plate for 2 hours before observation under the ×10 and ×40 magnifications of the microscope.

RESULTS

The Histological Analysis of the Various Organs Yields the Following Results

The Brain: Histological sections of the brain tissue showed glial cells and intervening neurons on a neutrophil background. There were some widening of the Robin - Virchow spaces (spaces surrounding blood vessels). This is indicative of cerebral oedema (fluid accumulation), which could have been caused by any mild injury.

<table>
<thead>
<tr>
<th>Lab No/ Code</th>
<th>Surface appearance</th>
<th>Colony form</th>
<th>Culture result (ZN-microscopy)</th>
<th>Opacity</th>
<th>Pigmentation</th>
<th>Culture appearance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1078</td>
<td>Rough rosette</td>
<td>Speedy raised</td>
<td>+ ve pinkish short rods</td>
<td>Translucent</td>
<td>Non-pigmented</td>
<td>Confluent</td>
</tr>
<tr>
<td>912</td>
<td>Smooth colonies</td>
<td>Speedy raised</td>
<td>+ ve pinkish short rods</td>
<td>Opaque</td>
<td>Pigmented</td>
<td>Confluent</td>
</tr>
<tr>
<td>954</td>
<td>Rough rosette</td>
<td>Speedy raised</td>
<td>+ ve pinkish short rods</td>
<td>Translucent</td>
<td>Non-pigmented</td>
<td>Confluent</td>
</tr>
<tr>
<td>472</td>
<td>Rough rosette</td>
<td>Speedy raised</td>
<td>+ ve pinkish short rods</td>
<td>Opaque</td>
<td>Non-pigmented</td>
<td>Buff-raised</td>
</tr>
<tr>
<td>2058</td>
<td>Smooth colonies</td>
<td>Speedy raised</td>
<td>+ ve pinkish short rods</td>
<td>Opaque</td>
<td>Pigmented</td>
<td>Confluent</td>
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<tr>
<th>Conc. Levels</th>
<th>Sensitivity/Resistant pattern of Mycobacterium tuberculosis at different concentration levels</th>
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<tbody>
<tr>
<td>Bacterial density</td>
<td>0.003g/ml</td>
</tr>
<tr>
<td>High 10⁻³ Low 10⁻³</td>
<td>High 10⁻³ Low 10⁻³</td>
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<tr>
<td>R R R R R R S S S S S S S S S S</td>
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The Kidney: Histological sections of the renal tissue showed intact glomeruli and tubules. No inflammation was seen.

The Liver: Histological sections of liver tissue showed normal hepatocytes arranged in plates, radiating towards the central veins. No inflammation was seen.

The Lungs: Histological sections of lung tissue showed alveoli with intact air spaces. No inflammation was seen. This result was the same for all subsequent sacrificial exercises, i.e. after 4 days, 2 weeks and 3 weeks.

DISCUSSION

In 1993, the World Health Organization (WHO) declared the rising incidence of tuberculosis a global public health emergency [10]. Since then, the disease has advanced, now causing an estimated 2 million deaths annually. The toxic effects of orthodox drugs in humans, the development of physiological resistance by *Mycobacterium tuberculosis* and high cost paved way for natural remedies as a reasonable alternative.

Natural products have been used for centuries in treating human diseases and they contain components of therapeutic value. Natural products are environmentally safer, easily available, cheap and have a long tradition in Africa. WHO advocates that claims of ailment remedies by traditional practitioners should be subjected to scientific and systematic evaluation, in order to fulfill its aims in making health care available to all.

The acceptance of traditional medicine as an alternative form of health care in Nigeria and the development of microbial resistance to the available antimicrobial agents stimulated this work.

In this study, the antimicrobial effect and safety (toxicity) of a popular cough remedy, alum, used in folk medicine was evaluated against *Mycobacterium tuberculosis*. The results showed that at the highest concentration of 0.003g/ml, *M. tuberculosis* was resistant to the alum extract, but susceptible to the control or standard drug (streptomycin) at the same concentration.

The result of this study confirms the work of [8,11], which stated that alum was not suitable as a drug for tuberculosis even though [12,13] and [14] reported that it had some antimicrobial effect. The ineffectiveness of (alum) at the normal concentration (0.003g/ml) used for the treatment of cough in folk medicine invalidates its use.

The histological analysis of the various organs (brain, kidney, liver and lungs) of mice administered with different doses of the test substance showed
no inflammation. Statistical analysis of the weight of the experimental animals compared with those of the controls showed no significant weight difference and no mortality was recorded throughout the experimental process.

The result of this study shows that alum is ineffective in the treatment of tuberculosis despite its wide use in folk medicine. The histological analysis of the various organs however suggests that alum is relatively safe when consumed orally.

REFERENCES