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Evaluation of the antimicrobial effects of copper oxide nanoparticles combined with Astragalus and Garlic extracts on *Mycobacterium tuberculosis* in a mouse model

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Extended Abstract

Introduction: Tuberculosis (TB) remains one of the most significant global health threats, with *Mycobacterium tuberculosis* being the causative agent. Despite advancements in medical treatment, TB continues to present challenges, particularly due to the emergence of drug-resistant strains. This study investigates the antimicrobial effects of a novel combination of garlic (*Allium sativum*) and astragalus (*Astragalus tragacantha*) extracts, enhanced with copper oxide (CuO) nanoparticles, on *Mycobacterium tuberculosis*. The motivation behind this study is to explore alternative, potentially more effective treatments for TB, considering the limitations of current therapies and the rise of multidrug-resistant TB strains. This study evaluates the synergistic antimicrobial action of these natural extracts in combination with CuO nanoparticles in a controlled mouse model.

Methods: The experimental setup involved the extraction of garlic and astragalus, followed by the synthesis of CuO nanoparticles. The plants were sourced from certified suppliers, and their identification was confirmed through herbarium samples. The extraction process involved preparing dried powders of garlic and astragalus, which were then processed using hydroalcoholic methods. The copper oxide nanoparticles were synthesized by adding copper sulfate to the extracts, followed by heating and centrifugation. The antimicrobial properties of the mixture were tested using the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) methods.

For in vivo testing, 20 Wistar rats were randomly divided into different groups, including control and treatment groups. The animals were inoculated with *Mycobacterium tuberculosis* and treated with varying doses (50 mg/kg and 100 mg/kg body weight) of the extract mixture containing CuO nanoparticles over a 21-day period. Tuberculin tests were conducted to evaluate the effect of the treatment on the progression of the infection.

Results: The in vitro testing showed that the garlic and astragalus extracts containing CuO nanoparticles were effective against *Mycobacterium tuberculosis*. The minimum inhibitory concentration (MIC) for the extract mixture was determined to be 50%, while the minimum bactericidal concentration (MBC) was 100%. CuO nanoparticles alone exhibited a MIC of 25% and an MBC of 25%. The combined extract with nanoparticles showed strong antibacterial activity, especially at concentrations of 12.5%, 25%, 50%, and 100%. The results also indicated a synergistic effect, as evidenced by a fractional inhibitory concentration (FIC) index of 0.75, which confirmed the enhanced antimicrobial activity when the extracts were combined with CuO nanoparticles. In the animal model, following the induction of tuberculosis, rats treated with the extract mixture at a dose of 100 mg/kg body weight showed a significant reduction in the size of the tuberculin test response. In half of the animals, the test results were negative, indicating a reduction in infection. The group receiving the lower dose (50 mg/kg) did not show significant improvement, and therefore, this dosage was excluded from further analysis.

Discussion: The results of this study support the potential of combining natural plant extracts with nanotechnology to combat drug-resistant *Mycobacterium tuberculosis*. The antimicrobial properties of garlic and astragalus, both of which have been traditionally used for their medicinal benefits, were significantly enhanced by CuO nanoparticles. These nanoparticles are known to generate oxidative stress that damages bacterial cells, which, when combined with the bioactive compounds in the extracts, produces a potent synergistic effect. The results from the animal model demonstrated the therapeutic potential of this combination, as evidenced by the reduction in tuberculin test responses. These findings align with previous studies that have highlighted the antimicrobial efficacy of CuO nanoparticles against various bacterial infections. Furthermore, the combination of garlic and astragalus extracts with CuO nanoparticles appears to be a promising approach for developing alternative treatments for tuberculosis, especially in the context of drug-resistant strains. However, there are limitations to the study, including the lack of direct comparison with conventional TB treatments and the challenge of translating animal model results to human treatment regimens. Further research, including clinical trials, is necessary to fully evaluate the safety and efficacy of this treatment in humans.

Conclusion: The combination of garlic and astragalus extracts with CuO nanoparticles shows promising antimicrobial effects against *Mycobacterium tuberculosis* in both in vitro and in vivo models. The synergistic effect observed in this study suggests that this formulation could be a potential candidate for developing new treatments for tuberculosis, especially in cases involving drug-resistant strains. Further studies are needed to optimize the dosage and to conduct clinical trials to validate the safety and effectiveness of this approach in human populations.

Keywords: Garlic extract, Astragalus extract, Copper oxide nanoparticles, *Mycobacterium tuberculosis*