Role of Testosteronthiosemicarbazone as an Antibacterial Agent

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Abstract

A ligand of testosteronthiosemicarbazone was prepared, the chemical characteristics of this ligand are: yellow powder, soluble in alcohol and its melting point is 100 – 102 °C.

The sensitivity of some species of pathogenic bacteria including Escherichia coli, Proteus vulgaris, Klebsiella pneumonia, Pseudomonas aeruginosa, Staphylococcus aureus, and Streptococcus feacalis to different concentrations of prepared testosteronthiosemicarbazone (0.05 g/ml, 0.1 g/ml, 0.25 g/ml, 0.5 g/ml, and 1 g/ml) was tested to find out the role of testosteronthiosemicarbazone as an antibacterial agent.

The experimental results of bacterial sensitivity to different concentrations of testosteronthiosemicarbazone, showed a significant sensitivity of all tested bacteria to this ligand at concentration starting from 0.5 g/ml, to 1 g/ml except Pseudomonas aeruginosa, and Streptococcus feacalis which they exhibited their significant sensitivity to the ligand at concentration starting from 0.25 g/ml, and 1 g/ml respectively.

Keywords: testosteronthiosemicarbazone, ligand, chelating agent, antibacterial agent.

دور التيستوستيرون ثايوسيميكاربزون كمادة مضادة للنمو البكتيري

الخلاصة

تم تحضير الخلب الجديد تيستوستيرون ثايوسيميكاربزون، و كانت الخصائص الكيميائية تيستوستيرون ثايوسيميكاربزون: مسحوق اصفر اللون ، قابل للذوبان في الكحول ، و درجة انصهاره تتراوح بين 100 – 102 °م.

تم قياس حسّاسية كل من الانواع البكترية الممرضة Escherichia coli و Proteus vulgaris و Klebsiella pneumonia و Pseudomonas aeruginosa و Staphylococcus aureus و Streptococcus feacalis إلى تراكيز مختلفة من التيستوستيرون ثايوسيميكاربزون (0.05 غم/مل و 0.1 غم/مل و 0.25 غم/مل و 0.5 غم/مل و 1 غم/مل) لغرض اختبار دور التيستوستيرون ثايوسيميكاربزون كمادة مضادّة للبكتريا.

بينت النتائج المختبرية حسّاسية كل الانواع البكترية المستخدمة في التجربة إلى التيستوستيرون ثايوسيميكاربزون ابتداء من تركيز 0.05 غم/مل الى التركيز الاعلى 1 غم/مل عدا *Pseudomonas aeruginosa* و *Streptococcus feacalis* التي اظهرت حساسية الى التيستوستيرون ثايوسيميكاربزون ابتداء من تركيز 0.25 غم/مل و عند التركيز 1 غم/مل على التوالي.

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Introduction

Over the past several years, the medical community has become increasingly concerned over the ability of certain bacteria to develop resistance to antibiotics [1,2]. Accordingly, there is a danger of losing the battle against certain pathogens (organisms causing diseases) by using the antibiotics in the treatment [1].

The development of drug resistance in human pathogens against commonly used antibiotics has necessitated a search for new antimicrobial substances from other sources including plants or synthesis of chemical compounds [3,4].

Thiosemicarbazone, a chemical compound, has received considerable attention in view of its variable bonding modes, promising biological implication, structural diversity, and ion-sensing ability [5, 6, 7, 8].

Another chemical compound, testosterone, is a steroid hormone [9, 10]. The role of testosterone as an antimicrobial agent has been proved [11].

Current study deals with the synthesis of a chemical compound, testosteronthiosemicarbazone, and studying the antibacterial activity of different concentrations of testosteronthiosemicarbazone against some tested bacterial species.

Materials and Methods

Synthesis of Testosteronthiosemicarbazone:

Hot ethanol solution of thiosemicarbazide (1.82g, 0.02 mol), and ethanolic solution of testosterone (2.26g, 0.02 mol) were mixed in the presence of few drops of concentrated hydrochloric acid (Fluka) with constant stirring. This mixture was refluxed for 3 hours. The completion of the reaction was confirmed by the TLC (Thin Layer Chromatography). The reaction mass was evaporated on a rotator evaporator. The product was filtered, washed with cold ethanol, and dried under vacuum over P_4O_{10}. [12]. (All reagents were obtained from Fluka).

Antibacterial Activity Test:

The antibacterial activity of testosteronthiosemicarbazone against different species of bacteria, *Escherichia coli*, *Proteus vulgaris*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Streptococcus feacalis* (the bacterial species are obtained from the labs of Biotechnology Department/ School of Applied Science at University of Technology) has been examined after calculating the bacterial growth to give 2×10^8 cell ml^{-1} that matches to 0.2 OD when read its turbidity at 600 nm wavelength [13]. 100 µl of each bacterial species was inoculated in tubes containing 5 ml of nutrient broth (HIMIDIA) and different concentrations of testosteronthiosemicarbazone (0.05, 0.1, 0.25, 0.5, and 1 g/ml). The tested tubes and control tubes were incubated at 37°C for 24 hr. Then the antibacterial activity and the MIC (minimum inhibitory concentration which inhibits bacterial growth at low concentration) [14, 15] of testosteronthiosemicarbazone was recorded by measuring the turbidity at 600 nm wavelenghth against blank. A triple reading has been done for each test.

Statistical Analysis:

The results were expressed as (mean ± SD). t-test was used to compare the growth of each bacterial species between control group and each of test tubes that contain the different concentrations of testosteronthiosemicarbazone.

The threshold of significance was chosen as ($P < 0.05$) [16]. The Statistic – Microsoft Excel has been used.

Results and Discussion

testosteronthiosemicarbazone has been prepared. After filtration, washing, and drying the product to form powder, the chemical characteristics of testosteronthiosemicarbazone powder were determined as: yellow powder, soluble in alcohol and its melting point is 100 – 102 °C [12].

The antibacterial activity of five different prepared concentrations of testosteronthiosemicarbazone (0.05, 0.1, 0.25, 0.5, and 1 g.ml) against *Escherichia coli*, *Proteus vulgaris*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Streptococcus feacalis* was recorded after 24 hr. of incubation. The results were analyzed statistically and compared with the control of each of selected bacterial species to find out the significant differences in the bacterial growth after treatment with testosteronthiosemicarbazone.

Figure 1 shows the mean absorbances of the growth of selected bacteria in controls and the test tubes that are treated with different concentrations of testosteronthiosemicarbazone. The results indicated a significant effect of testosteronthiosemicarbazone on decreasing the bacterial growth at concentration starting from 0.5 g/ml, to 1 g/ml except *Pseudomonas aeruginosa*, and *Streptococcus feacalis* which their growth has been decreased significantly at concentration starting from 0.25 g/ml, and 1 g/ml respectively as compared with their controls $p < 0.05$ (Figure 1), while the decreasing of the growth of selected bacteria was not confirmed significantly at concentrations lower than those mentioned above as compared with controls $p > 0.05$ (Figure 1). Accordingly, the MICs of testosteronthiosemicarbazone are 0.25 g/ml for inhibiting the growth of *Pseudomonas aeruginosa*, 0.5 g/ml for inhibiting the growth of *Escherichia coli*, *Proteus vulgaris*, *Klebsiella pneumonia*, and *Staphylococcus aureus*, and 1 g/ml for *Streptococcus feacalis* growth inhibition.

The inhibitory action of testosteronthiosemicarbazone is attributed to its chelating properties. It is a ligand which worked as chelating agent. The chelating agent has a tendency to chelate metal ions [17, 18, 19, 20]. It is suggested that this ligand might chelate metals necessary for the enzymatic catalysis in bacterial cell which might play a vital role in disrupting the metabolic pathways, or the biosynthesis of DNA [21, 22]. The chelating potency of this compound may depend upon the nature of the heteroatomic ring and the position of attachment to the ring as well as the form of thiosemicarbazone moiety [23]. Its flexibility, selectivity, and sensitivity towards the central metal atom, and similarities with natural biological substances, due to the presence of imine group (-N=CH-) might reflex its effect on the biological activity [22, 23].

The results of this report are in agreement with the study of (Alamiery, A.A,H., and Juwaied, A.A., 2009) [22]. They reported a considerable antibacterial activity of a prepared

ligand, hydrazinecarbothioamide against *Staphylococcus aureus, E. coli, Proteus vulgaris, Pseudomonas, and Klebsiella pneumonia*. They supposed that the antibacterial activity might be brought by chelating properties of the prepared ligand.

Conclusion

The experimental results indicate the role of prepared ligand, tetosteronthiosemicarbazone as an antibacterial agent by inhibiting the growth of all tested bacterial species, *Escherichia coli, Proteus vulgaris, Klebsiella pneumonia, Pseudomonas aeruginosa, Staphylococcus aureus,* and *Streptococcus feacalis*. The antibacterial activity of this lignad might be represented by its action as chelating agent which might chelate metals necessary for the enzymatic catalysis needed for living of bacterial cell.

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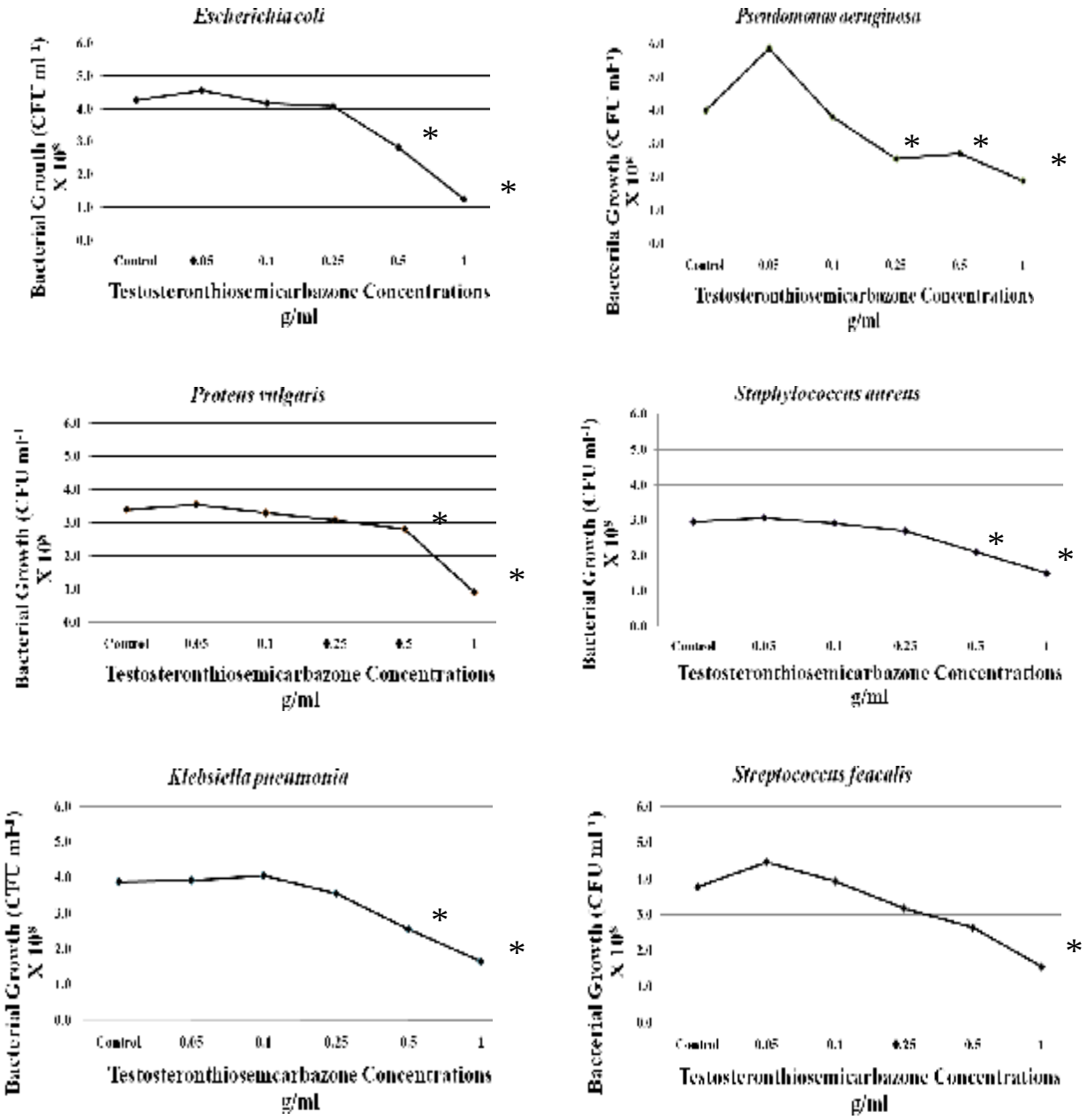
Figure(1).Antibacterial Activity of Testosteronthiosemicarbazone against the Growth of Some Bacterial Species



*: $P < 0.05$ (vs. control group)