TÜBİTAK

Turkish Journal of Veterinary and Animal Sciences

http://journals.tubitak.gov.tr/veterinary/

Short Communication

Turk J Vet Anim Sci
(2017) 41: 302-305

doi:10.3906/vet-1510-24

An investigation of antibacterial effects of steroids

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Received: 08.10.2015 • **Accepted/Published Online:** 17.02.2016 • **Final Version:** 19.04.2017

Abstract: In this study, the effects of steroid substances such as corticosterone, progesterone, estrone, beta-sitosterol, and stigmasterol on some bacteria were investigated. In the research, standard strains of the gram-positive bacteria *Bacillus subtilis* (lab isolate) and *Staphylococcus aureus* (ATCC 6538) and the gram-negative bacteria *Escherichia coli* (ATCC 25922), *Pasteurella multocida* (ATCC 12945), and *Pseudomonas aeruginosa* (ATCC 9027) were used. A modified broth dilution method was applied for determining the minimal inhibitory concentration (MIC) of the steroids. Mueller Hinton broth (Becton Dickinson, 211443) was used to prepare dilutions and as a medium. At the end of the study, the MIC of corticosterone for *P. multocida* and the MIC of beta-sitosterol for *S. aureus* were both determined as 32 µg/mL. However, other steroids showed no effects on the bacteria. These steroids did not appear to have widespread antibacterial properties.

Key words: Corticosterone, progesterone, estrone, beta-sitosterol, stigmasterol, antibacterial

Steroids (cholesterol and ergosterol) are components of cell membranes. There are important sterols like cardenolide-digoxigenin and bufadienolide-scillarenin, which have an effect on the heart. Fusidic acid, a steroid obtained from the fungus *Fusidium coccineum*, has antimicrobial characteristics and is used to treat infections caused by gram-negative and gram-positive bacteria (5–10 mg/kg 3 times per day). Alfaxolone and alfadolone are synthetic steroids developed in this way for general anesthesia (1).

Soybeans are a major source of beta-sitosterol and stigmasterol, which play a role in the growth of plant cells. Beta-sitosterol is added to products like margarine and corn oil. Fats like these have an antilipidemic effect because they reduce the absorption of cholesterol through the intestines (they cause less lipidemia than normal fats). Stigmasterol acts as an antioxidant. It also reduces the risk of prostate cancer (2,3).

It is known that plant-based sterols (stigmasterol) have a negative effect on the absorption of other steroids by the intestines (they also prevent the biosynthesis of cholesterol). Stigmasterol, campesterol, and beta-sitosterol have been demonstrated to inhibit the D24-reductase enzyme in Caco-2 and HL-60 cells. It has been found that stigmasterol inhibits the activity of liver 3-hydroxy-3-methylglutaryl-coenzyme A reductase and cholesterol 7a-hydroxylase (since the latter is a weak substrate, and since inhibition is attributed to the *7A1* gene). These effects reduce the level of cholesterol in the plasma (2).

The antitumor effect has only been procured with the administration of biogenic polyamine (4). Stigmasterol and spermine conjugate, which is a polyamine, can exhibit an antibacterial effect on *S. aureus* even at low concentrations. Some conjugates of steroids and polyamines have been shown to have cytotoxic effects (they are ineffective on HeLa but effective on malignant CEM cells and fibroblasts) (5).

The body synthesizes a large number of steroid-based substances whose chemical structures are very similar. Since newly synthesized and natural steroids resemble cholesterol, it is thought that they compete with it for the receptors. Not only can this prevent the normal development of the cell membrane, it can also disrupt cell integrity and permeability. It is thought that this is the reason why cholesterol derivatives can have an antibacterial effect. This view is strengthened by the fact that estrogens have an antibacterial effect (6). Due to the increase of resistance to antibiotics, the study of the antibacterial effects of new

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steroid derivatives is very important from the perspective of medical treatment. When we consider their mechanisms of effect, newly synthesized or developed cholesterol derivatives have the potential of being used as antibacterial drugs. Furthermore, determining the antibacterial effects of steroids with different chemical structures may make it possible to produce effective groups of steroids and to identify priorities in new synthetic pathways.

The goal of this study was to investigate the antibacterial effects of the animal-based steroids corticosterone, progesterone, and estrone, as well as the plant-based steroids beta-sitosterol and stigmasterol.

The corticosterone (CAS: 50-22-6), progesterone (CAS: 0130), estrone (CAS: 53-16-7), beta-sitosterol (CAS: 9889), and stigmasterol (CAS: 83-48-7) used in the study were purchased from the company Sigma. The substances were dissolved in a mixture of ethanol and water. The study used standard strains of *Bacillus subtilis* (lab isolate), *Staphylococcus aureus* (ATCC 6538), *Escherichia coli* (ATCC 25922), *Pasteurella multocida* (ATCC 12945), and *Pseudomonas aeruginosa* (ATCC 9027). Dilution of the bacteria was performed with a Mueller Hinton broth (Becton Dickinson, 211443), and the bacteria were grown in the same medium.

A modified version of the broth microdilution method reported by Wiegand et al. (7) and Ericsson and Sherris (8) was used to identify the minimum inhibitory concentration (MIC) levels of the steroids in the study. A Mueller Hinton broth medium was used to dilute and grow the bacteria (Table). Fifty microliters of the test substance solution, prepared at a density of 64 mg/L, was placed into the first well of a sterile microplate. The concentration was then quickly diluted to 32–0.0625 µL/mL using the broth. A bacteria suspension with a 0.5 McFarland turbidity standard was then prepared from colonies in a 24-h fresh bacteria culture. The final inoculum concentration was set to 5×10^5 cfu/mL. Equal amounts of bacterial suspension were added to the wells (containing the active substance diluted to 1/100), which contained a series of the substances diluted with Mueller Hinton broth (50 µL in each well). The tests were performed twice, with separate positive and negative control test tubes for each solution. To ensure sterility in the test, broth alone was placed in a well, while bacterial inoculum with broth was placed in another well as a control sample for growth. The mediums were incubated at 37 °C for 24 h.

The MIC levels of the tested substances were calculated by using a spectrophotometer (Epoch, Sn: 1504121/USA) at a wavelength of 620 nm to measure the density of the wells before and after incubation.

The effects of corticosterone, progesterone, estrone, beta-sitosterol, and stigmasterol on the gram-positive bacteria *B. subtilis* and *S. aureus* and the gram-negative bacteria *E. coli*, *P. multocida*, and *P. aeruginosa* have been tested with a modified microdilution method (7,8).

Corticosterone only prevented the growth of *P. multocida* at a concentration of 32 µg/mL. Beta-sitosterol was only observed to inhibit the growth of *S. aureus* at a concentration of 32 µg/mL, while its effect on other bacteria was undetermined. However, progesterone, estrone, and stigmasterol did not prevent growth at any concentration with the bacteria that were tested.

Efforts to synthesize new drugs are very important because resistance to antibacterial agents is significantly

Table. Preparation of the agents used in the broth dilution method.

Step	Antimicrobial concentration (mg/L)	Antimicrobial concentration step	Antimicrobial amount (mL)	Broth amount (mL)	Antimicrobial concentration obtained (µg/mL)	Final concentration in the test (µg/mL)
1	640	Stock	1	9	64	32
2	64	From the 1st step	1	1	32	16
3	64	From the 1st step	1	3	16	8
4	64	From the 1st step	1	7	8	4
5	8	From the 4th step	1	1	4	2
6	8	From the 4th step	1	3	2	1
7	8	From the 4th step	1	7	1	0.5
8	1	From the 7th step	1	1	0.5	0.25
9	1	From the 7th step	1	3	0.25	0.125
10	1	From the 7th step	1	7	0.125	0.0625

increasing at the present time. It has been possible to significantly accelerate drug synthesis by making changes to a molecule that has a prototypical effect. Some derivatives of steroids like fusidic acid have antibacterial effects. Studies have shown that of the natural steroids, estrogen has a higher antibacterial effect than progesterone (6).

The agar diffusion method has been used to show that follicular fluid obtained from humans is effective against 30 strains of *S. agalactiae*. Approximately 8.3% of the fluid samples were found to have a strong effect, 53.3% had a moderate effect, and 38.3% had no effect. The suggestion has been made that this effect was due more to the lysozymes contained in the fluid rather than the steroids (9). Human follicular fluid obtained from patients using oocyte aspiration for in vitro fertilization and embryo transfer has been shown to be effective against gram-positive and gram-negative microorganisms, as well as against *Candida albicans*. This inhibition has been attributed to the progesterone and the lysozyme enzymes that it contains (10). A study was performed on the antibacterial effects of estrogen and progesterone in the uterus of mares; it was reported that the estrous cycle did not have any effect on streptocidal activity or immunoglobulin levels (11). Results of that study show a similarity to the current research.

Steroid–polyamine conjugates have a negative effect on cell proliferation. It has been demonstrated that when sterols are conjugated with polyamines, which bind to DNA, they are effective against gram-positive and -negative bacteria, fungi, and protozoa (12). It has been determined that squalene and spermine obtained from sharks have a bactericidal effect. Similarly, a conjugate of stigmasterol and spermine has been found to be effective on *S. aureus* (5). Dexamethasone–spermine, an aminosterol conjugate, has been demonstrated to have a bactericidal effect on *S. aureus* and *P. aeruginosa*. It has been determined that this conjugate prevents inflammation that involves molecules like interleukin-6 (IL-6), lipopolysaccharide (LPS), and IL-8. This is why the suggestion has been made that conjugates may be a treatment option for bacterial infections involving inflammation (13). It has been shown that a testosterone–vitamin B_1 conjugate inhibits the growth of *S. aureus* (MIC 2.57×10^{-1} mmol), *E.coli*, and *K. pneumonia* (MIC 1.48×10^{-1} mmol) (14). A pregnenolone–carbamazepine conjugate has been found to have MIC levels on *Proteus mirabilis* that are comparable to cefotaxime, gentamicin, and ciprofloxacin (15). A pregnenolone–danazol conjugate has been demonstrated to have an effect of disrupting the cell membrane of bacteria like *S. aureus*, *E. coli*, and *K. pneumonia* by means of the quaternary amine group carried by the conjugate (16). It is apparent from various studies that both steroids and polyamines are important for creating a cytotoxic effect (4). The fact that polyamines have a high antibacterial effect and exhibit toxicity with low immunological side effects supports the idea of using them as medication in the future (17).

The plant *Veronica anthelmintica*, a member of the family Compositae, was used to isolate a new steroid called vernoanthelsterone A and 5 glycosides. It has been suggested that forskolin, one of these compounds, can be used to increase the concentration of 17-beta-estradiol (EC_{50} 56.95 μg/mL). It can be suggested that these kinds of aromatase enzyme agonists may be beneficial in diseases caused by estrogen deficiency. On the other hand, it is known that aromatase inhibitors are used for breast tissue cancers in which estrogen is effective. It has been determined that compounds isolated from these plants have an effect on *B. cereus*, *S. aureus*, *B. subtilis*, and *E. coli* that is comparable to that of ampicillin (at 12.5–100 μg/mL). The sensitivity of bacteria to these compounds varies from 3.15 to 250 μg/mL (18). *Nerium oleander* (white oleander)'s roots have been used to chemically procure a cardenolide called 12b-hydroxy-5β-carda-8,14,16,20,22-tetraenolide. Studies have been carried out on the antibiotic characteristics of this substance as well as its cardiac effects, which are similar to those of digitoxin. Some compounds extracted from plants have been shown to have moderate effects on *B. subtilis*, *B. cereus*, *E. coli*, *P. aeruginosa*, *S. paratyphi*, and *Shigella dysenteriae* (19). *Dioscorea bulbifera* L. var *sativa* (Dioscoreaceae) is a plant that grows wild in Bangladesh that has bitter tubers and is used by local people for diseases like leprosy and tumors. Two diterpenoids of the clerodane series have been isolated from this plant. A study was conducted on these substances using the agar diffusion and dilution methods to investigate their antibacterial effects on *P. aeruginosa*, *E. coli*, *K. pneumoniae*, *S. aureus*, *S. typhi*, *S.* Paratyphi A, and *S.* Paratyphi B. Both substances were shown to have a significant effect on *P. aeruginosa* and a low effect on *S. typhi*, *S.* Paratyphi A, and *S.* Paratyphi B (20).

When we examine the relationship between composition and effect, it is notable that corticosterone has 4 reactive regions, progesterone and estrone have 2, and beta-sterol and stigmasterol have 1 each. Therefore, it can be suggested that it should be possible to rank their strengths and spectrums of effect according to this relationship. However, this may not always be possible because of differences in the biochemical makeup of microorganisms. Studies have been done that suggest that the antibacterial effects of steroids may be caused by the peroxide and vinyl bonds in their structures (5). The mechanism of the effect of the sterols can be explained by the fact that they are similar to sterols that are normally used in the cells, and that they replace these substances in the cell membrane. The antibacterial activity of aminosterols can be explained by the fact that they imitate

polymyxin B. It is thought that the effective component in sterol–polyamine conjugates is the hydrophilic chain that is due to the hydrophobic structure. For this reason, some studies suggest that the bile acid–ethylamine conjugate is much more active. It is thought that efficacy could be increased by extending the side-chain (17).

In conclusion, this study found that corticosterone (for *P. multocida*) and beta-sitosterol (for *S. aureus*) inhibited the growth of certain microorganisms. It can be said that when the steroids are analyzed according to the number of bacteria affected and the effective concentrations that were identified, they are not superior to current drugs with regard to antibacterial effect. Furthermore, the number of different bacteria and steroids that were investigated does not provide clear information about the sensitivity of gram-positive and gram-negative bacteria to the steroids that were used. More extensive pharmacodynamics and pharmacokinetic studies need to be conducted to shed light on this subject.

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