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The Effect of Different Hormones and Antibiotics on Activity of AST Enzyme and its Isozymes in Wistar Rats

Minoo Azani, Ali Asghar Moshtaghie & Ali Asghar Rastegari

ABSTRACT

***Background*:** One of the valuable tests for diagnosis of cardiovascular and liver diseases is measuring of AST activity. One of the main enzymes of transaminases group is aspartate aminotransferase. Previous Studies have shown that some alteration may occur in mitochondria function as the result of different disease or taking different medication; these changes in mitochondrial and cytosolic AST isozymes can be the sign of disorders. According to the role of steroid hormone in induction of its effects on protein synthesis genes, this study is conducted to shed some light on mechanisms and the interference of steroid hormones and antibiotics.

***Materials, Methods & Results*:** In this study, male Wistar rats were injected intramuscularly with Testosterone, progesterone and estradiol; while tetracycline and streptomycin injections were intraperitoneal. Testosterone, progesterone and estradiol injections were carried out in a short-term (15 days) and long-term (45 days) periods. Steroid hormones were dissolved in sesame in a way that by each injection, 0.2 mL sesame oil (containing specific amount of hormone) was injected to the rat. Control group was kept in the same condition except that their sesame oil injection contained no hormone. Tetracycline and Streptomycin injection was carried out for 5 days at 7 am and pm, at 50 mg/kg dosage intraperitoneally. In short- and long-term periods, rats were divided into four groups of 6-member. The concentrations were the same in the periods and 0.2 mL sesame oil was injected intramuscularly. 1 mg testosterone, 12 mg progesterone and 0.2 mg estradiol were intramuscularly injected to rats in group 2, 3 and 4, respectively [10]. Rats were divided into 9 six-member groups as follows: Group 1: intraperitoneal injection of 0.2 mL physiological serum; group 2: injection of 1 mg testosterone; group 3: injection of 1 mg testosterone + 50 mg/kg streptomycin; group 4: injection of 1 mg testosterone + 50 mg/kg tetracycline; group 5: injection of 0.2 mg estradiol; group 6: injection of 0.2 mg estradiol + 50 mg/kg streptomycin; group 7: injection of 0.2 mg estradiol + 50 mg/kg tetracycline; group 8: injection 50 mg/kg streptomycin; and group 9: injection of 50 mg/kg tetracycline. Serum concentration of AST enzyme was measured at the end of each period and the data were compared by SPSS software. all three steroid hormones had no significant impact on AST activity in short term. However, a significant effect was observed in long term in mean AST activities of the 4 groups. The group injected by testosterone exhibited 9% increases in comparison with the control group. Antibiotic-administrated groups showed lower activities as compared with hormone-injected groups. Steroid hormones and testosterone can enhance AST activity, in short-term and long-term, respectively by induction of protein enzyme. The second test confirmed this theory as the antibiotics decreased the AST activity enhancement by testosterone.

***Discussion*:** Based on the present study, steroid hormones can enhance the aspartate aminotransferase activity; and antibiotics can decrease the level of this liver enzyme by inhibition of polypeptide synthesis on related genes. This reaction could be due to interference of hormones and antibiotics effect which hinders the hormone effect along with the drug to activate the protein synthesis process.

Keywords: steroid hormones, testosterone, progesterone, estradiol, AST.

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Department of Biochemistry, Falavarjan Branch, Islamic Azad University, Isfahan, Iran. CORRESPONDENCE: A.A. Moshtaghie [moshtaghie_a@iaufala.ac.ir -Tel: +98 (313) 374 20 134]. Department of Biochemistry, Falavarjan Branch, Islamic Azad University. 84515 Isfahan, Iran.

INTRODUCTION

Measurement of AST activity has been used as a valuable test for diagnosis of cardiovascular and liver diseases. One of the main enzymes of transaminases group is aspartate aminotransferase (EC 2.6.1.1) [16]. It has high concentration in heart, liver and skeleton muscles and low levels in kidneys, pancreas and erythrocytes. This enzyme has two mitochondrion and cytosolic isozymes. Clinical application of AST is mainly associated with evaluation of myocardial infarction (MI), liver and skeleton muscles cell disorders [10].

Steroid hormones can make some variations in the size, spatial structure and surface charge of the target cell which enable them to attach to chromatin and activate or deactivate special genes [9]. Estrogens are deactivated by biochemical reaction and the produced metabolites will dissolve along with composition with glucuronic acid and sulfuric acid and rapidly excreted by kidneys [7]. The most important Progesterone metabolite which is excreted by kidney is sodium Pregnanediol-20- glucuronide [6].

It has been well proven that tetracycline can prevent the protein synthesis in bacteria [2]. Streptomycin is among the Aminoglycosides which is a protein formation inhibitor [11]. Studies have shown that some alteration may occur in mitochondria function as the result of different disease or taking different medication; these changes in mitochondrial and cytosolic AST isozymes can be the sign of disorders. Regarding the role of steroid hormone in induction of its effects on protein synthesis genes, in this study the effect of steroid hormones, alone or in combination with Streptomycin and tetracycline, on AST enzymes and isozymes was investigated.

MATERIALS AND METHODS

Animals

In this study, Wistar rats (*Ratus norvegicus*) were used which were purchased from Pasture institute. They were then kept in animal cage of Islamic Azad University of Falavarjan; at temperature of 25 ± 3 and all animals consumed the foods in their cages.

Study groups

The applied rats were mature and male with the weight of 220 ± 10 g which were kept in 6-member groups.

Testosterone[1], progesterone[1] and estradiol[1] were injected intramuscularly; while tetracycline1 and streptomycin[1] injections were intraperitoneal. Testosterone, progesterone and estradiol injections were carried out in a short-term (15 days) and long-term (45 days) periods. Steroid hormones were dissolved in sesame in a way that by each injection, 0.2 mL sesame oil (containing specific amount of hormone) was injected to the rat. Control group was kept in the same condition except that their sesame oil injection contained no hormone.

Tetracycline and Streptomycin injection was carried out for 5 days at 7 am and pm, at 50 mg/kg dosage intraperitoneally [13,14].

In short- and long-term periods, rats were divided into four groups of 6-member. The concentrations were the same in the periods and 0.2 mL sesame oil was injected intramuscularly. 1 mg testosterone, 12 mg progesterone and 0.2 mg estradiol were intramuscularly injected to rats in group 2, 3 and 4, respectively [15].

Antibiotic injection period

Rats were divided into 9 six-member groups as follows:

Group 1: intraperitoneal injection of 0.2 mL physiological serum; *Group 2*: injection of 1 mg testosterone; *Group 3*: injection of 1 mg testosterone + 50 mg/kg streptomycin; *Group 4*: injection of 1 mg testosterone + 50 mg/kg tetracycline; *Group 5*: injection of 0.2 mg estradiol; *Group 6*: injection of 0.2 mg estradiol + 50 mg/kg streptomycin; *Group 7*: injection of 0.2 mg estradiol + 50 mg/kg tetracycline; *Group 8*: injection 50 mg/kg streptomycin; and *Group 9*: injection of 50 mg/kg tetracycline.

Blood sampling and laboratory measurements

At the end of injection period, the animals were first anesthetized by Ketamine[2] 10% along with xylasine[2] 2%, the blood was directly sampled from their heart by a 10-cc syringe (in some cases, the animals were fixed on the plate and their chest was opened and blood sampling was carried out form the heart) and transferred to tubes. 7 cc blood samples can be obtained from each healthy rat. Then the samples were kept for 1 h followed by 15 min centrifugation at the rate of 96 *g*. The serum was separated and rapidly transferred to freezer for further investigation.

The level of (SGOT) AST enzyme or Aspartate amino transaminase was measured by photometric method through application of an autoanalyzer[3].

For isolation of HMW and LMW isozymes, gel filtration along with trace buffer was employed [8]. After collection of fractions, SGOT of each fraction was measured and the curves were plotted in which the activity ranged along y-axis and number of fractions were on x axis.

Data analysis

ANOVA[4] test (SPSS v 21) was applied for comparison of the variables of control and case groups (progesterone, estradiol and testosterone injection). Comparison of the 9 groups (including control, testosterone, estradiol, tetracycline, streptomycin, testosterone+ tetracycline, testosterone+ streptomycin, estradiol + tetracycline and estradiol+ streptomycin) for investigation of the effect of antibiotics on enzyme activities was carried out by ANOVA test (SPSS v 21).

RESULTS

Effect of steroid hormones on activity of total AST in rats during a 15-day period

According to Table 1, there was no significant difference in short-term average of AST enzymes in the 4 studied groups [control, progesterone, estradiol and testosterone] ($P > 0.05$). Therefore, injection of three steroid hormones had no significant impact on AST enzyme activity in short-term, but an increase can be observed relative to control group in Figure 1. Testosterone injection has the highest impact on AST activity in short term. Compared to control group, testosterone, progesterone and estradiol groups had 35%, 26% and 24% increase, respectively.

Effect of testosterone on AST isozymes following 15-day intramuscular injection

To investigate the variation of AST isozymes in rat serums, samples were taken on Sephacryl S-300 column and 2 mL fractions were collected and measured. Figure 2 shows the variation of AST isozymes in rat serum during a 15-day period. Activity of HMW-AST showed a 64% increase relative to control sample at its activity peak. LMW-AST showed 48% increase.

Effect of steroid hormones on activity of total AST in rat serum during a 45-day period

According to Table 2, there was a significant difference in long-term mean of AST activity between the 4 groups ($P < 0.05$).therefore, long-term steroid hormone injections could have a significant impact on AST activities. Based on the results of post-hoc Bonferoni test, there was a significant difference in AST activity of only testosterone and progesterone groups ($P < 0.05$). Testosterone had the highest impact on AST activities in long-term.

Table 1. Descriptive indices for AST enzyme for different short-term steroid hormone injection.

Group	Number	Mean ± SD	Min	Max
Control (1)	6	127.17 ± 2.48	117	133
Testosterone (2)	6	172.01 ± 19.17	131	262
Progesterone (3)	6	160.17 ± 5.88	142	176
Estradiol (4)	6	158.51 ± 9.30	135	186

Table 2. Descriptive indices for AST enzyme for different steroid hormone injection in long-term.

Group	Number	Mean ± SD	Min	Max
Control (1)	6	118.83 ± 3.88	105	132
Testosterone (2)	6	129.08 ± 8.88	99.5	163
Progesterone (3)	6	98.16 ± 4.89	87.5	118
Estradiol (4)	6	118.25 ± 5.24	96.5	131

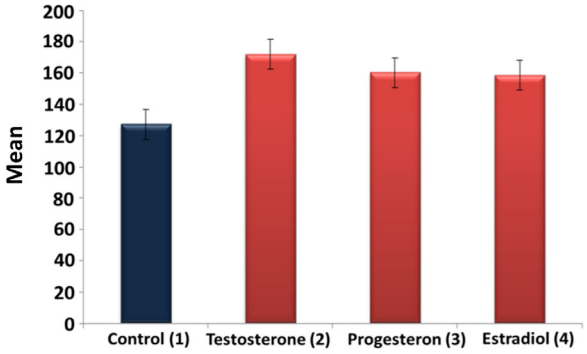

Figure 1. Mean AST activity increase in short term in different injection groups.

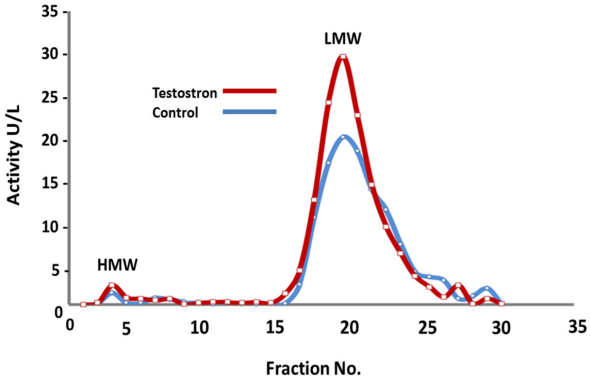

Figure 2. Effect of testosterone on activity of AST isozymes following intramuscular injection of testosterone at dosage of 1 mg for 15 days.

As Figure 3 shows, the testosterone group had a 9% increase in comparison with the control group while the progesterone and estradiol groups had 20% and 0.55% reduction, respectively.

Effect of testosterone on AST isozymes following 45-day intramuscular injection

Figure 4 shows activity of HMW-AST showed a 30% increase relative to control sample this increase was 42% for LMW-AST.

Effect of antibiotics on total AST activity of rat serum during a 5-day period

According to Table 3, there was no significant difference average of AST enzymes of 9 studied groups [control, progesterone, estradiol and testosterone hormones and tetracycline and streptomycin antibiotic] ($P > 0.05$). According to Figure 5, injection of testosterone +tetracycline had the highest impact on AST activity as compared by the control group.

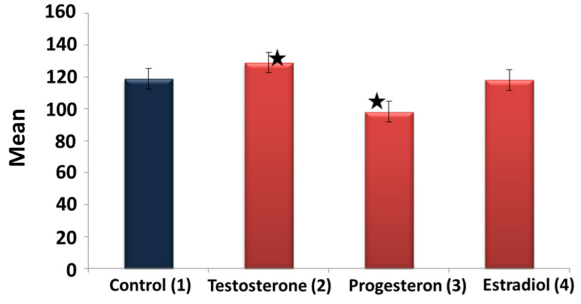

Figure 3. Comparison of mean AST activity in long-term for different injection groups.

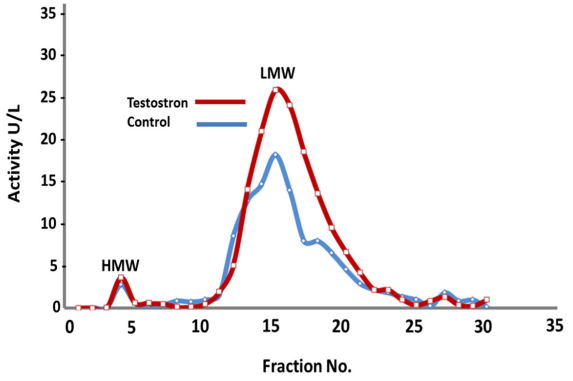

Figure 4. Effect of testosterone on activity of AST isozymes following intramuscular injection of testosterone at dosage of 1 mg for 45 days.

Table 3. Descriptive indices for AST enzyme for different antibiotic injection.

Group	Number	Mean ± SD	Min	Max
Control (1)	6	162.02 ± 6.52	132	176
Testosterone (2)	6	169.33 ± 20.25	128	259
Estradiol (3)	6	146.33 ± 11.65	115	193
tetracycline (4)	6	144.17 ± 5.67	119	158
Streptomycin (5)	6	151.33 ± 3.36	139	162
Testosterone+ tetracycline (6)	6	152.67 ± 5.48	126	190
Testosterone+ streptomycin (7)	6	164.33 ± 14.86	125	209
Estradiol +tetracycline (8)	6	143.01 ± 10.76	106	187
Estradiol+ streptomycin (9)	6	162.83 ± 6.43	135	181

Effect of testosterone and testosterone+ tetracycline on AST isozymes following intramuscular and intraperitoneal injections

Figure 6 shows variation of AST isozymes in antibiotic injection period following testosterone and testosterone+ tetracycline injections. In comparison with control group, HMW-AST and LMW-AST activities had 33% and 48% decrease in the testosterone+ tetracycline group, respectively. In the testosterone-treated group an increasing trend was observed.

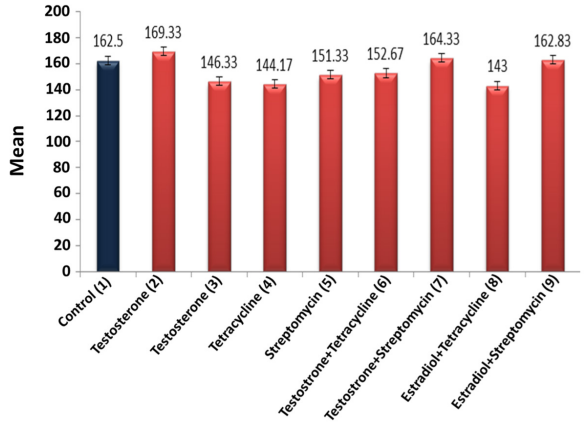

Figure 5. Mean AST enzyme in 9 studied groups.

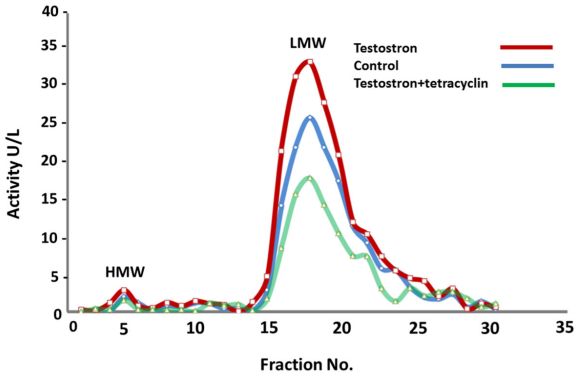

Figure 6. Variation of AST isozymes following 1 mg testosterone and 1 mg testosterone + 50 mg/kg tetracycline injection.

DISCUSSION

Our results showed that in a short-term period and compared to control group, all the groups including estradiol, progesterone and testosterone treated groups had 24%, 26% and 35% increase in their AST activities, respectively. Although these increases were not statistically significant among the groups ($P < 0.056$) but these mean values indicated increasing effect of hormones, especially testosterone, on AST activity. Study of Sanford *et al*. [13] showed that progesterone rapidly affected the regulation center of Endocrine glands and enzymic system of Hepatocytes within a week (short term) and resulted in 70% increase in enzymes activity as compared with the control group. These results reflect the effect of steroid hormones on liver enzymes in a short-term period. As testosterone and progesterone are among steroid hormones, by study of their short-term effects on hepatocytes intercellular mechanisms it can be concluded that these hormones affect the target cells more rapidly. In a way that these hormones can enter the nucleus by linking to cytoplasmic receptor protein and then attach to their specific receptors on the nucleus surface; this hormone-receptor complex would interact with gene sequence and induce its effect on protein synthesis genes and activate the translation trend of the involved genes and therefore increase the protein synthesis and enhance liver enzymes activities. Our results are in agreement with the work of Sanford *et al*. [13]. It has been anticipated that progesterone will have the highest impact on enzyme activities; however, our study showed that testosterone has a strong influence on hepatocytes and induces more variation in enzymic functions.

In the second round of experiments, blood sampling and analysis of AST enzyme were carried out 45 days after the hormones' injection (long-term period). Enzyme activity was measured by autoanalyzer and the results of different groups were compared with each other and control group. Results showed that there is a significant difference between the groups and control group. Estradiol and progesterone-treated groups showed 0.5% and 20% decrease as compared with the control group. Testosterone treatment resulted in 9% increase in AST enzyme which was statistically significant ($P < 0.05$). In the work of Fred *et al*. [4] with rats, it was revealed that AST enzymes had 3-7 fold increase after injection of glucocorticoids. This increase had direct relationship with effect period and type of injection. In a way that long-term subcutaneous injections after more than 73 h had increasing impact on enzymes and affect synthesis of enzyme proteins and increased their activities. Studies on the effect of hormones on activity of liver enzymes showed that

glucocorticoids (due to their spatial structure and surface charge) have the ability to bond with oppositely charged DNA surface and activate specific genes for production and copying enzyme proteins. This activation process took long time for having proper impact. Therefore, it has been observed that after 45 days, enzyme activities were increased which could be due to synthesis of enzyme proteins as suggested by Fred *et al*. [4].

Comparison of the short-term and long-term effects of steroid hormones on liver and aspartate aminotransferase activity showed that, due to their specific receptors, estradiol and progesterone affect the metabolism system of hepatocytes shortly after injection and increased aspartate aminotransferase activity (although very little) and it is expected to be excreted by urine after making their impacts. That's why they don't have significant long-term impact on AST increase in liver. On the other hand, it was observed that testosterone can alter aspartate aminotransferase activity in long-term. While short-term investigations showed that this hormone induced higher increase in AST activities; therefore it seems that testosterone could have both long- and short-term effect on hepatocytes. This hormone effect on the genes involved in AST protein synthesis is not time dependent and can impose its impact in different time periods.

Hormones and two types of antibiotics were injected to 9 groups of rats and their impacts on aspartate aminotransferase activity were investigated after 5 days. The findings indicated that testosterone-treated group had the highest rate of aspartate aminotransferase activity reflecting the effect of this hormone on synthesis of enzyme proteins. Other studied groups (estradiol, tetracycline, streptomycin, testosterone+ streptomycin, testosterone+ tetracycline, estradiol streptomycin and estradiol+ tetracycline) showed no significant increase in enzyme activity.

Akande *et al*. [1] investigated the effect on streptomycin and penicillin on liver enzymes of rabbit. They showed that high doses of these drugs will induce acute hepatitis in hepatocytes and significantly decrease aspartate aminotransferase activity and total serum protein.

Study of Rotov *et al*. [12] addressed the effect of antibiotics such as streptomycin and tetracycline on performance of liver enzymes. It was revealed that these antibiotics managed to decrease aspartate aminotransferase and alkaline phosphatase activities by inhibitory impact on ATSase and also intercellular mechanisms of hepatocytes.

In the work of Friedmaus *et al*. [5], it was indicated that liver enzymes are the function of variations of hepatocytes imposed by drugs. Antibiotics such as penicillin and streptomycin could disturb liver function and by reducing the protein level and also enzyme activities, they can act as an index for liver toxicity of these drugs in high dosage.

Comparison of our results with previous studies showed that antibiotics enter the liver as drugs with lipophilic structures. If they enter the body in overdoses, they will be identified as toxins by the liver and P450 cytochromes will be activated to fight with them. During this detoxifying process, activity of liver enzymes will be reduced as well. On the other hand, antibiotics are known as protein synthesis inhibitors; so if they enter the cells in high doses, they will be attached to 16srRNA subunits and interfere with protein and tRNA transcription and cause a reduction in enzyme protein synthesis and finally enzyme activity. In this regard, the observations of our study were in line with pervious works.

After purification and investigation of two serum isozymes of the testosterone-treated groups in short-term investigation, it can be concluded from Figure 2 that this hormone affected hepatocytes and AST enzyme which resulted in 64% increase in cytosolic isozyme (HMW-AST) relative to control group/ moreover, activity of mitochondrial isozyme (LMW-AST) of aspartate aminotransferase experienced a 48% increase relative to control sample.

Investigation of long-term effect of testosterone on aspartate aminotransferase iso-enzymes showed that after 45 days, this hormone increased cytosolic and mitochondrial isozymes of AST by 30% and 42%, respectively (Figure 4).

Comparison of control group with testosterone+ tetracycline-treated group indicated a 33% and 48% decrease in activities of cytosolic and mitochondrial AST isozymes, respectively. However, testosterone invoked increasing variations (Figure 6).

As function regulation of aspartate aminotransferase isozymes in the liver depends on calcium channels and calcium content of the cells, these isozymes will be disturbed under abnormal calcium levels on hepatocytes. These disturbances along with increase of

AST isozymes can be considered as the sign of damage to hepatocytes function and also isozymes [17].

Endo *et al*. [3] in their studies on rats observed that aspartate aminotransferase activity increases in mitochondrial section of hepatocytes following glutamate injection as a stimulus for increasing hepatocytes' metabolism. This increase was 20% and 75% for cytosolic and mitochondrial isozymes, respectively. Therefore, it can be concluded that glutamate stimulations on mitochondrion will accumulated the calcium ions which in turn will increase the activity of AST-related isozymes.

Comparison of the variations in cytosolic and mitochondrial isozymes of aspartate aminotransferase in testosterone-treated groups in short and long-term periods and also the impact of tetracycline showed that enzymes such as aspartate aminotransferase determine the liver function. These enzymes are themselves regulated by the mechanisms and alterations in their subunits. Therefore, any factors (chemical, physical, oxidative stress, etc.) which alter these isozymes activity, can change the enzyme activities and be a sign of disorder in hepatocytes' function.

CONCLUSIONS

Results of this study showed that steroid hormones in short term (and testosterone in long-term) can activate the responsible genes for synthesis, transcription and translation of involved genes and increase protein production and also enzyme activities, in particular cytosolic isozyme of aspartate aminotransferase. On the other hand, regarding the effect of antibiotics on protein production process in DNA and cells nuclei, application of tetracycline in this study showed expected results in line with enzyme activity reduction. By inhibition of protein production cycle on gene level, it weakened the effect of hormones on this cycle and therefore decreased the activity of AST. Therefore based on the literature and results of present study, it can be claimed that steroid hormones have enhancing effect on aspartate aminotransferase activity; and antibiotics will decrease the level of this liver enzyme by inhibition of polypeptide synthesis on related genes. This reaction could be due to interference of hormones and antibiotics effect which hinders the hormone effect along with the drug to activate the protein synthesis process.

MANUFACTURERS

[1]Merck KGaA. Darmstadt, Germany.

[2]Sigma-Aldrich Co. Rocklin, CA, USA.

[3]Tajhizat Sanjesh Co. Esfahan, Iran.

[4]IBM Corp. Armonk, NY, USA.

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Declaration of interest. The authors report no conflicts of interest. The authors alone are responsible for the content and writing of paper.

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