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Dear

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Title: **Comparative Study of Testosterone, Spironolactone, Docetaxel and Their Combination Effect on Prostate Cancer Cell Line LNCaP**

We pleased to inform you that your above mentioned article has been accepted for publication in NeuroQuantology.

Regards

Editor
NeuroQuantology

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وزارة التعليم العالي والبحث العلمي

جامعة بابل

كلية الطب

دراسة خارج الجسم للتحقق من نشاط السبيرونولاكتون المضاد لتكاثر خط خلايا سرطان البروستات

رسالة

مقدمه إلـــى مجلس كليـــة الطـــب / جامعـــة بابـــل

كجزء من متطلبـات نيل درجـة الماجستير في الادوية/ ادوية وسموم

من قبل

رواء عادل عليوي ناصر

(بكلوريوس صيدلة ٢٠١٤)

إشراف

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الخلاصة

تم تنفيذ الجزء العملي لهذه الدراسة في مختبر أبحاث الدراسات العليا / فرع الأدوية والسموم / كلية الطب / جامعة بابل خلال الفترة من ديسمبر 2021 إلى أبريل 2022. يعد سرطان البروستاتا ثاني أكثر أنواع السرطانات شيوعًا والسبب الرئيسي الخامس للوفاة بالسرطان بين الرجال في جميع أنحاء العالم ، وبحسب تقدير عام 2018 فان هناك نحو 1276000 حالة سرطان جديدة و 359000 حالة وفاة في عام 2018.

تم في هذه الدراسه اجراء خمسة تجارب مختبرية على خط خلايا البروستاتا السرطانية LNCaP حيث تم في التجربة الاولى تعريض الخلايا لكل من هرمون التستوستيرون و سبيرونولاكتون والعلاج الكيميائي الدوسيتاكسيل بشكل منفصل حيث تم استخدام التراكيز التالية (1000 ، 500 ، 250 ، 125 ، 62,5 و 31,25 مايكروغرام / مل) لكل مادة. وفي التجربة الثانية تم تعريض الخلايا الى مزيج من السبيرونولاكتون و التستوستيرون التي تحتوي على تركيز متساوٍ لكل مادة (500 + 500 ، 250 + 250 ، 125 +125 ، 62,5 + 62,5 ، 31,25 + 31,25 ، 15,625 + 15,625 مايكروغرام / مل). اما في التجربة الثالثة فقد تم تعريض الخلايا لمزيج من تركيز ثابت للدوسيتاكسيل(500 مايكروغرام / مل) مع سلسلة من التراكيز المخففة للسبايرونولاكتون (500 ، 250 ، 125 ، 62,5 ، 31,25 و 15,625 مايكروغرام / مل). وفي التجربة الرابعة تم تعريض الخلايا لمزيج من تركيز ثابت للسبايرونولاكتون(500 مايكروغرام/مل) مع سلسلة من التراكيز المخففة للدوسيتاكسل (500 ، 250 ، 125 ، 62,5 ، 31,25 و 15,625 مايكروغرام / مل). واما في التجربة الخامسة فقد تم تعريض الخلايا لمزيج من التستوستيرون مع السبيرونولاكتون و دوسيتاكسيل بتراكيز متساوية لكل منهم حيث تم استخدام سلسلة من التراكيز المخففة لهذا المزيج (500 + 500 + 500 ، 250 + 250 + 250 ، 125 + 125 + 125 ، 62,5 + 62,5 + 62,5 ، 31,25 + 31,25 + 31,25 ، 15,625 + 15,625 + 15,625 مايكروغرام / مل).

لقد تم قياس حيوية الخلايا في التجارب اعلاه عن طريق اختبارالسمية الخلوية التترازوليوم (MTT) والذي يعتمد على قياس شدة اللون بواسطة قارئ ELISA وتم ذلك بعد فترة حضانة 24 و 48 ساعة في التجارب الاربعة الاولى في حين تم حضن الخلايا لمدة 48 ساعة في التجربة الخامسة. حيث أظهرت نتائج الدراسة انخفاض عدد الخلايا الحية بشكل ملحوظ مع زيادة تركيز السبيرونولاكتون والتستوستيرون.

لقد أظهرت النتائج أن التراكيز 1000،125،250،500 و 62,5 مايكروغرام / مل من التستوستيرون تسببت في حصول زيادة معنوية (P >0.05) في حيوية الخلايا LNCaP مقارنة بمجموعة السيطرة بعد 24 ساعة من الحضانة. اما بعد 48 ساعة من الحضانة أظهرت النتائج ان التراكيز 125 ، 62,5 و 31,25 مايكروغرام / مل تسببت في حصول زيادة غيرمهمة معنوياً (P>0.05) في حيوية خلايا LNCaP ، بينما 250 مايكروغرام / مل تسبب في حصول انخفاضًا معنويًا (p < 0.05) في حيوية خلايا LNCaP ،اما التراكيز 500 و 1000 مايكروغرام / مل فقد سببا انخفاضًا معنويًا كبيراً (p<0.001) في حيوية خلايا LNCaP عند مقارنته بمجموعة السيطرة.

وكذلك أظهرت النتائج أن جميع تراكيز السبيرونولاكتون سبب في حصول زيادة غير مهمة معنوياً (p>0.05) في حيوية خلايا LNCaP مقارنة بمجموعة السيطرة بعد 24 ساعة من الحضانة. اما بعد 48 ساعة من الحضانة فان سبيرونولاكتون بتركيز 62,5 مايكروغرام / مل تسبب في حصول زيادة معنوية (P<0.05) في حيوية خلايا LNCaP بالمقارنة مع مجموعة السيطرة. بينما التراكيز 31,25 ، 125 و 1000 مايكروغرام / مل فقد سببت في حصول انخفاضاً معنوياً في حيوية هذه الخلايا. لم تظهر النتائج حصول فرق مهم إحصائيا في حيوية خلايا LNCaP بين مجموعتي السيطرة و تلك التي تعرضت للتركيز 250 مايكروغرام / مل بعد 48 ساعة من الحضانة.

لقد بينت النتائج ان التركيز 250 مايكروغرام / مل من الدوسيتاكسيل تسببت في حصول انخفاضًا معنويًا (p<0.05) في حيوية خط خلايا LNCaP اما التراكيز 500 و 1000 مايكروغرام / مل فقد تسببت في حصول انخفاضًا معنويًا كبيرا (p<0.001) في خط خلايا LNCaP ، فيما لم يكن الفرق مهم إحصائيا (0.05<p) في حيوية خلايا LNCaP بين مجموعة السيطرة وكل من المجاميع التي تعرضت للتراكيز 31,25 ، 62,5 و 125 مايكروغرام / مل بعد 24 ساعة من الحضانة. أما بعد 48 ساعة من الحضانة فقد أظهرت النتائج أن جميع تراكيز الدوسيتاكسيل تسببت في حصول انخفاضًا معنويًا كبيرا(0.001>p) في حيوية خط خلايا LNCaP مقارنة بمجموعة السيطرة.

اما عند تعريض خط خلايا LNCaP لمزيج مكون من تراكيز متساوية لكل من السبيرونولاكتون و التستوستيرون فقد أظهرت النتائج حصول انخفاضا معنوياً كبيراً (0.001<P) في حيوية هذه الخلايا عند تعريضها للتراكيز 31,25 ، 62,5 ، 500 و 1000 مايكروغرام / مل. في حين تسببت

التراكيز 125 و 250 مايكروغرام / مل بحصول زيادة معنوية (P<0.05) في حيوية هذه الخلايا بالمقارنة مع مجموعة السيطرة بعد فترة حضانة لمدة 24 ساعة. أما بعد 48 ساعة من الحضانة فقد بينت النتائج حصول انخفاض معنوي (P<0.05) في حيوية خط خلايا LNCaP بتركيز 31,25 مايكروغرام / مل وكان هناك انخفاض غير مهم معنوياً (p>0.05) في حيوية الخلايا عند التركيز 500 مايكروغرام / مل بالمقارنة مع مجموعة السيطرة.

اما عند تعريض خط خلايا LNCaP لمزيج مكون من تراكيز مختلفة من السبيرونولاكتون مع تركيز ثابت للدوستاكسل(500 مايكروغرام/مل) فقد أظهرت النتائج أن تراكيز المزيج 500 + 125 ، 500 + 250 و 500 + 500 مايكروغرام / مل قد تسببت في حصول زيادة معنوية (P <0.05) في حيوية خط خلايا LNCaP ، في حين أن تراكيز المزيج 500 + 15,625 ، 500 + 31,25 و 500 + 62,5 مايكروغرام / مل لم تتسبب في حصول فروقات مهمة إحصائيا في حيوية خط خلايا LNCaP بالمقارنة مع مجموعة السيطرة بعد مدة حضانة 24 ساعة. فيما أظهرت النتائج أن جميع تراكيز المزيج أعلاه قد تسببت في حصول انخفاضًا معنويًا كبيرا (p <0.001) في حيوية خط خلايا LNCaP مقارنة بمجموعة السيطرة بعد 48 ساعة من الحضانة.

اما عند تعريض خط خلايا LNCaP لمزيج مكون من تراكيز مختلفة من الدوسيتاكسل مع تركيز ثابت من السبيرونولاكتون (500 مايكروغرام/مل) فقد أظهرت النتائج أن التركيز 31,25+500 مايكروغرام / مل فقد تسبب في حصول زيادة غير معنوية (P>0.05) في حيوية خط خلايا LNCaP، بينما التركيز 15,625+500 مايكروغرام / مل فقد تسبب في حصول نقص غيرمهم معنوياً (P>0.05) في حيوية خلايا LNCaP . في حين أن تراكيز المزيج 62,5 +500 ، 125 +500 و 250+500مايكروغرام / مل قد تسببت في حصول انخفاضًا معنويًا (P <0.05) في حيوية خط خلايا اما التركيز 500 + 500 مايكروغرام/مل فقد تسبب في حصول انخفاضاً معنوياً كبيرا (p<0.001) في حيوية هذه الخلايا مقارنة بمجموعة السيطرة بعد 24 ساعة من الحضانة.

أظهرت النتائج أيضًا ان جميع تراكيز المزيج أعلاه وبعد فترة حضانة 48 ساعة قد تسببت في حصول انخفاضا معنويا كبيرا (P <0.001) في حيوية خط خلاياLNCaP مقارنة بمجموعة السيطرة.

فيما يخص تعريض خط خلايا LNCaPلعدة تراكيز من مزيج يحتوي كل منها على تركيز متساوي لكل من التستوستيرون ،السبايرونولاكتون والدوسيتاكسل وبعد مدة حضانة 48 ساعة وبالمقارنة مع مجموعة السيطرة فقد بينت النتائج أن المزيج المكون من التركيز 62,5 و125 مايكروغرام/ مل والمزيج المكون من التركيز 250 و 500 ميكروغرام / مل لكل مكون من المكونات أعلاه قد تسببت في حصول انخفاضا معنويا ($P<0.05$) وانخفاضا معنويا كبيرا($p<0.001$) على التوالي في حيوية خط خلايا LNCaP وقد كان التأثير الأكثر سمية على هذه الخلايا هو للمزيج الحاوي على التركيز500 مايكروغرام / مل من كل مكون. أما فيما يخص تاثير المزيج المكون من التركيز 15,625 و 31,25 مايكروغرام / مل فقد أظهرت النتائج حصول انخفاض غير مهم إحصائيا ($p>0.05$) في حيوية هذه الخلايا.

يستنتج من نتائج الدراسة الحالية أن للتراكيز العالية من التستوستيرون (وبعد فترة حضانة 48 ساعة) والسبايرونولاكتون تأثيرا مثبطا لحيوية خط خلايا سرطان البروستات LNCaP وللتراكيز العالية من الدوسيتاكسيل بعد فترة حضانة 24 ساعة وجميع تراكيزه بعد فترة حضانة 48 ساعة على التوالي تأثيرا مثبطا لحيوية خط خلايا سرطان البروستات.LNCaP وان لمزيج كل من السبايرونولاكتون مع التستوستيرون و السبايرونولاكتون (بعدة تراكيز) مع الدوسيتاكسل (تركيزثابت) و الدوسيتاكسل (بعدة تراكيز) مع السبايرونولاكتون (تركيزثابت) تأثيرا مثبطا لحيوية خط خلايا سرطان البروستات .LNCaP وأيضا لمزيج التستوستيرون ،السبايرونولاكتون والدوسيتاكسل تأثيرا مثبطا لحيوية خط خلايا سرطان البروستات LNCaP والتاثير الافضل كان للمزيج ذو التركيز500 مايكروغرام/مل لكل منهم .

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Conclusions

At the end of this study, the conclusions were:

1. Testosterone has concentration and time dependent proliferative effect on the prostate cancer LNCaP cell line.

2. Spironolactone at high concentrations have antiproliferative effect on the prostate cancer LNCaP cell line.

3. Docetaxel has concentration and time dependent antiproliferative effect on the prostate cancer LNCaP cell line.

4. Docetaxel plus spironolactone have antiproliferative effect on the prostate cancer LNCaP cell line.

Recommendations

The main recommendations were:

1. In vivo studies on lab animals are recommended.

2. Investigate the effect of spironolactone on other androgen dependent cancer cell line.

Summary

The practical work of the present research study was performed at the Postgraduate student's research laboratory/Department of Pharmacology and Toxicology / College of Medicine / University of Babylon during the period from December 2021 –April 2022.

The aim of the present study is to evaluate the effect of spironolactone alone and in combination with testosterone and ⁄or docetaxel on prostate cancer cell line LNCaP.

The following experiments were done to investigate:

1. Effect of docetaxel, spironolactone and testosterone on the viability of LNCaP cell line after 24 and 48 hours of incubation. The LNCaP cell line was exposed to serial dilutions of each testosterone, docetaxel and spironolactone (1000, 500, 250, 125, 62.5 and 31.25 µg/ml) separately. The cytotoxicity was assessed by using MTT assay.

2. Effect of spironolactone in combination with testosterone on the viability of LNCaP cell line after 24 and 48 hours of incubation. The LNCaP cell line was exposed to serial dilutions of spironolactone plus testosterone combination which contain equal concentration of each agent (500+500, 250+250, 125+125, 62.5+62.5, 31.25+31.25, 15.625+15.625 µg/ml). Then the cytotoxicity was assessed by using MTT assay.

3. Effect of different concentrations of spironolactone in combination with constant concentration (500 µg/ml) of docetaxel on the viability of LNCaP cell line after 24 and 48 hours of incubation. The LNCaP cell line was exposed to the combination of docetaxel (500 µg/ml) plus serial dilutions of

spironolactone (500, 250, 125, 62.5, 31.25 and 15.625 µg/ml). Then the cytotoxicity was assessed by using MTT assay.

4. Effect of different concentrations of docetaxel in combination with constant concentration (500µg/ml) of spironolactone on the viability of LNCaP cell line after 24 and 48 hours of incubation. The LNCaP cell line was exposed to combination of spironolactone (500 µg /ml) plus serial dilutions of docetaxel (500, 250, 125, 62.5, 31.25 and 15.625 µg /ml). Then the cytotoxicity was assessed by using MTT assay

5. Effect of the spironolactone, testosterone and docetaxel combination on the viability of LNCaP cell line after 48 hours of incubation. The LNCaP cell line was exposed to serial dilution of spironolactone plus testosterone and docetaxel combination which contain equal concentration of each agent (500+500+500, 250+250+250, 125+125+125, 62.5+62.5+62.5, 31.25+31.25+31.25, 15.625+15.625+15.625 µg /ml). Then the cytotoxicity was assessed by using MTT assay.

Cell viability was measured in the above experiments by tetrazolium cytotoxicity test (MTT), which is based on chromatography by ELISA reader. This was done after 24 and 48 hours incubation in the first four experiments, while the cells were incubated for 48 hours in the fifth experiment. The results of the study showed that the number of live cells decreased significantly with the increase in the concentration of spironolactone and testosterone.

The results showed that the concentrations of 1000, 125, 250, 500 and 62.5 µg/ml of testosterone caused a significant increase ($P > 0.05$) in the viability of LNCaP cells compared to the control group after 24 hours of incubation. After 48 hours of incubation, the results showed that concentrations 125, 62.5 and

31.25 µg/ml caused a significant (P>0.05) increase in the viability of LNCaP cells, while 250 µg/ml caused a significant decrease ($p < 0.05$) in the viability of LNCaP cells, while the concentrations of 500 and 1000 µg/ml caused a highly significant decrease ($p<0.001$) in the viability of LNCaP cells when compared to the control group.

Also, the results showed that all spironolactone concentrations caused a significant ($p>0.05$) increase in the viability of LNCaP cells compared to the control group after 24 hours of incubation. After 48 hours of incubation, spironolactone at a concentration of 62.5 µg/ml caused a significant ($P<0.05$) increase in the viability of LNCaP cells compared to the control group.

While the concentrations 31,25, 125 and 1000 µg/ml caused a significant decrease in the vitality of these cells. The results did not show a statistically significant difference in the viability of LNCaP cells between the two control groups and those that were exposed to a concentration of 250 µg/ml after 48 hours of incubation.

The results showed that a concentration of 250 µg/ml of docetaxel caused a significant ($p<0.05$) decrease in the viability of the LNCaP cell line, while concentrations of 500 and 1000 µg/ml caused a significant decrease ($p<0.001$) in the LNCaP cell line. , while the difference was not statistically significant ($p>0.05$) in the viability of LNCaP cells between the control group and each of the groups exposed to concentrations 31,25, 62,5 and 125 µg/ml after 24 hours of incubation. After 48 hours of incubation, the results showed that all concentrations of docetaxel caused a significant decrease ($p<0.001$) in the viability of LNCaP cell line compared to the control group.

When the LNCaP cell line was exposed to a mixture of equal concentrations of spironolactone and testosterone, the results showed a significant ($P<0.001$) decrease in the vitality of these cells when exposed to concentrations 31,25, 62.5, 500 and 1000 μg/ml. While the concentrations 125 and 250 μg/ml caused a significant increase ($P>0.05$) in the viability of these cells compared to the control group after a 24-hour incubation period. After 48 hours of incubation, the results showed a significant ($p<0.05$) decrease in the viability of LNCaP cell line at a concentration of 31.25 μg/ml, and there was a significant ($p>0.05$) decrease in the viability of cells at a concentration of 500 μg/ml in comparison. with a control group.

When the LNCaP cell line was exposed to a mixture of different concentrations of spironolactone with a fixed concentration of docetaxel (500 μg/ml), the results showed that the concentrations of the mixture 500 + 125, 500 + 250 and 500 + 500 μg/ml caused a significant increase ($P < 0.05$) in the viability of the LNCaP cell line, while the concentrations of the mixture 500 + 15,625, 500 + 31,25 and 500 + 62.5 μg/ml did not cause statistically significant differences in the viability of the LNCaP cell line compared to the control group after The incubation period is 24 hours. While the results showed that all concentrations of the above mixture caused a significant ($p<0.001$) decrease in the viability of LNCaP cell line compared to the control group after 48 hours of incubation.

When the LNCaP cell line was exposed to a mixture of different concentrations of docetaxel with a fixed concentration of spironolactone (500 μg/ml), the results showed that the concentration 31,25500+ μg/ml caused a non-significant increase ($P>0.05$) in the vitality of the line. LNCaP cells, while the concentration of 15,625 + 500 μg/ml caused a non-significant ($P>0.05$) decrease in the viability of LNCaP cells. Whereas, the concentrations of the

mixture 62.5 + 500, 125 + 500 and 250 + 500 µg / ml caused a significant decrease ($P < 0.05$) in the viability of the cell line, while the concentration 500 + 500 µg / ml caused a highly significant decrease ($p<0.001$) in the viability of these cells compared to the control group after 24 hours of incubation.

The results also showed that all concentrations of the above mixture and after incubation period of 48 hours caused a significant decrease ($P<0.001$) in the viability of LNCaP cell line compared to the control group.

With regard to exposing the LNCaP cell line to several concentrations of a mixture, each of which contains an equal concentration of testosterone, spironolactone and docetaxel, and after an incubation period of 48 hours, and in comparison with the control group, the results showed that the mixture consisting of the concentration 62.5 and 125 µg/ml and the mixture consisting of the concentrate 250 and 500 µg/ml for each of the above components caused a significant ($P<0.05$) and significant ($p<0.001$) decrease, respectively, in the viability of the LNCaP cell line, and the most toxic effect on these cells was for the mixture containing the concentration 500. µg/ml of each component. As for the effect of the mixture consisting of 15,625 and 31.25 µg/ml, the results showed a statistically non-significant decrease ($p>0.05$) in the vitality of these cells.

The conclusion of our study: The development of prostate cancer cell is suppressed by high testosterone concentration whereas cell growth is stimulated by low testosterone concentration. The viability of LNCaP cell line decreased when spironolactone concentration increased to 1000µg/ml. Docetaxel suppresses the development of cultured prostate cancer in a dose- independent way after 48 hours of incubation.

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ADT	Androgen deprivation therapy
AR	Androgen receptor
AS	Active surveillance
ATM-Chk2	ataxia-telangiectasia mutated checkpoint pathway
BID	twice daily
CRPC	Castration resistant prostate cancer
DDW	deionized distilled water
DHT	Dihydrotestosterone
DMSO	Dimethyl sulfoxide
DRE	digital rectal examination
EDTA	Ethylenediaminetetraacetic acid
FBS	Fetal bovine serum
IC50	The inhibitory concentration of 50% of cells
LHRH	Luteinizing hormone-releasing hormone
mg	milligram
µg	microgram
ml	milliliter
MRI	magnetic resonance imaging

mTOR	mammalian Target Of rapamycin
NKG2D	Natural Killer Group 2D
PBS	phosphate buffer saline
PC3	Prostate cancer cells
PCa	Prostate cancer
PET	positron emission tomography
PI3K/Akt	phosphatidylinositol 3-kinases / Protein kinase B (PKB, or Akt)
PSA	Prostate-specific antigen
RARP	robot-assisted radical prostatectomy

Combined Effect of Testosterone and Spironolactone on Proliferative Activity of Prostate Cancer Cell Line (Lncap)

Rawaa A. Alawi AL Khafaji[1*], Entisar J. Hamad AL-Mukhtar[2], Rana A. Ghaleb[3]

Abstract

Background: Prostate cancer, also known as malignant neoplasms of the prostate, typically begins in the glandular tissue. While these malignancies, which are mostly adenocarcinomas, are usually indolent, prostate cancers that are extremely aggressive and have a poor prognosis are discovered in a small percentage of men. and strongly affected by steroid hormones, especially androgens. Testosterone hormone interferes with cancer cell invasion into surrounding tissue by cell migration, which is the first stage of tumor metastasis.

Aim of the study : was to evaluate the results of testosterone, spironolactone, and testosterone plus spironolactone (on the viability) percentage of LNCaP cell line.

Materials and Methods: LNCaP, a human prostate cancer cell line that is sensitive to androgen, was employed; it was subjected to various concentrations of testosterone, spironolactone, and testosterone plus spironolactone combination. After 24 and 48 hours of incubation, the MTT test was used to analyze their influence on the viability proliferative or antiproliferative effect of the LNCaP cell line.

Results: At low concentrations, testosterone increased the viability of LNCaP cells significantly ($p>0.05$), whereas after 48 hours, it caused a high significantly $p < 0.001$ decreased viability at high concentrations. Spironolactone treatment increased the viability of the LNCaP cell line significantly $p>0.05$, but it decreased) the viability of the LNCaP cell line at high concentrations. The reduction in cell viability caused by testosterone plus spironolactone is significantly high $P< 0.001$.

Conclusion: On the LNCaP cell line, high testosterone and spironolactone concentrations and spironolactone plus testosterone had an antiproliferative effect.

Key Words: Testosterone, Spironolactone, LNCaP cell line, prostate cancer

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Introduction

Prostate cancer, also known as malignant neoplasms of the prostate, typically begins in the glandular tissue. Prostate cancers that are particularly aggressive and have a bad prognosis are discovered in a certain population of men, despite the fact that these tumours, primarily adenocarcinomas, are frequently indolent.[1] More than 80% of cases are significantly correlated with age, with elderly men (> 65 years of age) experiencing the highest prevalence. Prostate cancer is the fifth most common cause of death worldwide and the second most frequently diagnosed malignancy in men. Despite this, only 10% of men with prostate cancer die as a result of their condition. 39 % of men aged 70 to 79 and 43 % of men over 80 are found to have prostate cancer. Sex hormones (testosterone) are thought to affect prostate cancer risk. Some men may have higher levels of testosterone than others, the hormone responsible for male sex. Because a boost in testosterone levels causes the prostate gland to enlarge, men who use testosterone therapy are more likely to develop prostate cancer.[3] Aldosterone binding to the mineralocorticoid receptor is inhibited by the aldosterone antagonist

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spironolactone. Additionally, This medication has an antiandrogenic effect, which may be caused by, among other things, a peripheral androgen antagonistic effect. In vivo studies suggest that spirolactone prevents [3H] 5 alpha-dihydrotestosterone [3H] DHT from binding to the cytosolic and nuclear receptor of the rat ventral prostate. For the particular S cytosolic receptor, spirolactone can outbid [3H] DHT. Additionally, testosterone's production is hampered by spirolactone. [4]

Materials and methods

In RPMI-1640 media with the addition of (penicillin) (100 U/ml), (streptomycin) (100 g/ml), and 5% (fetal bovine serum) at 37°C in 5% CO2, the LNCaP cell line was cultured. These cancer cells are androgen-dependent, human prostate (adenocarcinoma) cells originating from lymph node metastases. At a density of 5*105 cells/ml, (LNCaP) cells were planted in (tissue culture) 96-well plates before 24 hours of the treatment with either testosterone (Testopel) 100mg/1ml, spironolactone (Aldacton) 25mg, or testosterone plus spironolactone combination treatments. Distilled water 5ml was used to dissolve spironolactone and placed in sonicator at 37 C0. Sonication accelerates the dissolution of a solid into a liquid by agitating particles in a solution with sound waves[5], which is then diluted with complete growth media) to obtain (final concentrations) of (1000, 500, 250, 125, 62.5, 31.25 µg/ml) for spironolactone, also similar concentrations were prepared for testosterone. The combination of spironolactone plus testosterone was prepared by adding equal concentrations of each agent (500+500, 250+250, 125+125, 62.5+62.5, 31.25+31.25, 15.625+15.625 µg/ml). Subsequent that, 200 µl of each concentration was poured into each well and left to incubate for another 24 or 48 hours. After 24 and 48 hours exposure period, the wells were cleaned with 200 µl of (sterile PBS. The MTT assay was used to investigate the effects of testosterone, spironolactone and combinations of testosterone plus spironolactone on the growth of the (LNCaP) cell line. The MTT assay determines how quickly a (tetrazolium) salt is converted into a (formazan) product in the cell (purple color). The opacity of the purple color is (directly proportional) to the number of live cells; this may be evaluated using (spectrophotometry) and gives a relative estimate of cell viability. Three replicates for each concentration was considered. Microsoft Office Excel 2010 was used to collect and analyze all of the data. A one-way Anova test was used to examine the differences between each treated group and the control group. P-values below 0.05 and above 0.001 were considered statistically significant and highly significant, respectively.

Results

Results showed that the concentrations 1000,500,250,125 and 62.5 µg/ml of testosterone cause significant ($p<0.05$) increase in the viability of (LNCaP)cell line in comparison to the control group after 24 hours of incubation.

After 48 hours of incubation results showed that the testosterone concentrations 125, 62.5 and 31.25 µg/ml cause insignificant ($P>0.05$) increase in the viability of LNCaP cell line, while testosterone concentration 250 µg/ml cause significant ($p < 0.05$) decrease and 500 and 1000 µg/ml cause highly significant ($p<0.001$) decrease in the viability of LNCaP cell line when compared to the (control group) as shown in (figure 1).

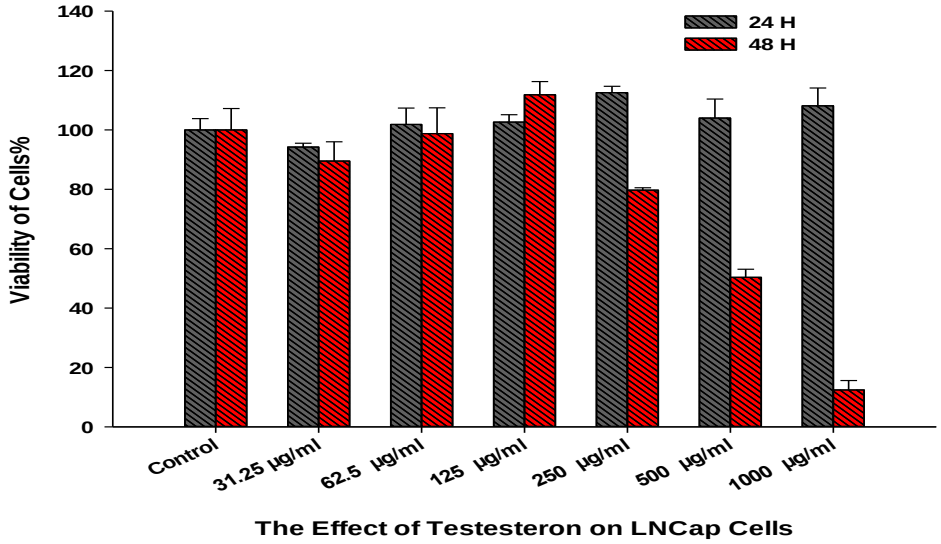

Figure 1: Effect of (Testosterone) on the Viability Percentage of (LNCaP) cell line after 24 and 48 hours of incubation

Results showed that all concentrations of spironolactone cause insignificant (p>0.05) increase in the viability of LNCaP cell line in comparison to the control group after 24 hours of incubation.

After 48 hours of incubation results showed that the spironolactone concentration 62.5 µg/ml compared to the control group, results in a significant (P<0.05) increase in cell viability. While at concentration 31.25, 125 and 1000 µg/ml causes a significant decrease in cells viability. There were (no significant) differences between the control group and 250 µg/ml groups for the same time of incubation as shown in (figure 2).

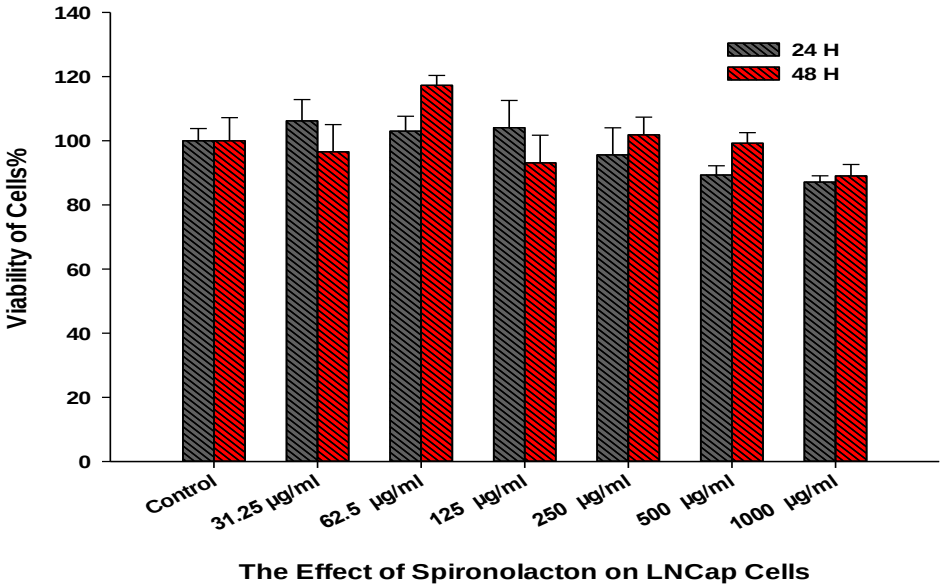

Figure 2: Effect of spironolactone on the viability percentage of LNCaP cell line after 24 and 48 hours of incubation.

The results showed that at concentrations of 31.25, 62.5, 500, and 1000 µg/ml, there was a highly significant drop in cell viability (P< 0.001). After 24 hours of incubation, cell viability increased significantly; (p> 0.05) at concentrations of 125 and 250 µg/ml in comparison to control

group. After 48 hours of incubation, there was a significant ($p>0.05$) drop in cell viability at concentrations of 31.25 g/ml and 500 g/ml when compared to the control group as shown in (figure 3).

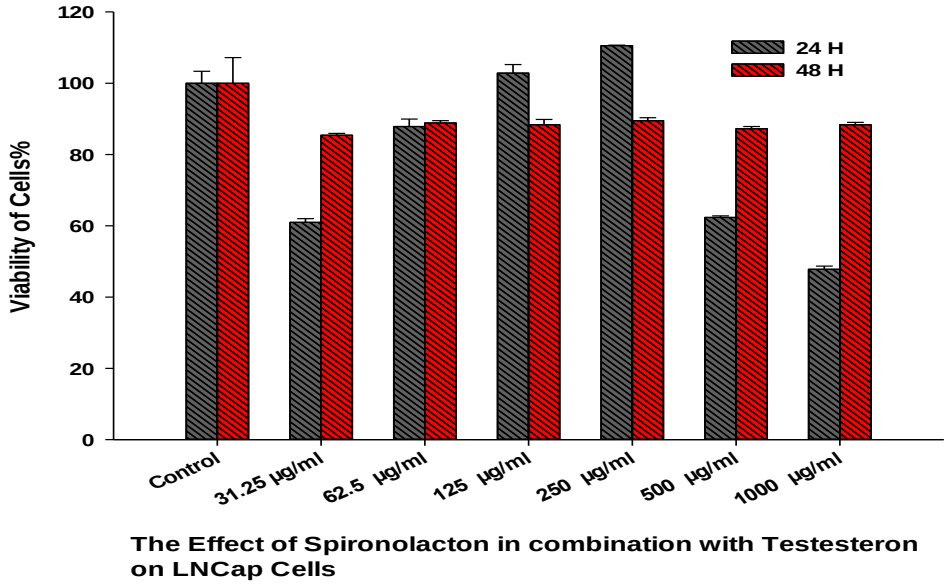

Figure 3: Effect of spironolactone plus testosterone combination on the viability percentage of LNCaP cell line after 24 and 48 hours of incubation.

Discussion

Regarding the role of testosterone in promoting or surpassing prostate cancer, evidences are inconsistent. Testosterone is not a tumor-promoting factor for prostate cancer, but it is possible that it caused prostate epithelial cells to develop hypersensitivity to androgens, giving them a selective growth advantage that led to a few of those cells progressing to malignancy.[6]

Through ligand-mediated activation of the androgen receptor (AR), testosterone increases the propagation of LNCaP cells, which drives prostate cancer progression .[7] The androgen receptor (AR) is a key player in the prostate, muscle, bone, and adipose tissue. Additionally, dysregulated AR activity is a catalyst for the onset and spread of prostate cancer (PCa). According to the androgen receptor, androgens promote LNCaP propagation via PI3K/Akt-independent activation of mTOR and subsequent post-transcriptional increases in cyclin D protein synthesis. [8]

There was an evident increase in cell proliferation at low concentrations and this rise remained largely consistent even as the hormone concentration increased. Morgentaler and Traish (2009) found that despite logarithmically higher testosterone concentrations, cell propagation increased gradually with rising testosterone at low concentrations, then plateaued with no further response. [9]

A study by Anagnostopoulou et al., (2013) stated that in animal models, testosterone has been shown to inhibit cancer cell proliferation by counteracting the proliferative effects of endogenous hormones like DHEA. [10]

Because very high testosterone levels inhibit the proliferation of prostate cancer cells, this effect could be used in prostate cancer treatment. However, these pathways have largely remained undiscovered at the molecular and cellular levels. As a result, the purpose of this study is to see how different testosterone concentrations affect the viability of (prostate cancer) cells. High testosterone has an inhibitory effect on cell viability in the LNCaP cell line due to androgenic receptor (AR) downstream signaling or (non-genomic) AR activity. Furthermore, hormonal activation of the androgen receptor causes the receptor to self-stabilize, resulting in an increase in AR activity. As a result, in clinical practice, a therapeutic decline in (androgen levels) is a clinical target that would result in a drop in AR activity and, as a result, a reduction in AR-driven prostate cancer progression. [11]

The viability of the LNCaP cell line increased after treatment with a low dosage of spironolactone, but

decreased significantly when the concentration was increased to 1000 µg/ml. These results agree with Isla, (2016) who found that taking spironolactone was linked to a decreased risk of prostate cancer in a retrospective, matched cohort study.[12] Also, this result agrees with Walsh, (1975) who found that spironolactone can suppress the level of plasma androgens by spironolactone. These findings could be attributed to the effect of spironolactone as an inhibitor of androgen synthesis, as found in a Walsh study in which men with metastatic prostate cancer who had been castrated received spironolactone. Due to spironolactone's capacity to inhibit the production of adrenal androgens, this study discovered that plasma levels of testosterone, androstenedione, and dehydroepiandrosterone were significantly decreased. [13]

Also, agree with Jody, 2020 who found that spironolactone because of its anti-androgenic properties binds non-selectively to other steroid receptors, including progesterone and androgen receptors. The investigators found that men who had ever used spironolactone had significant 17% decreased odds of PCa. The effect was strongest among current users of the drug, who had significant 23% decreased odds of PCa. Spironolactone exposure was significantly associated with 18% and 22% decreased odds of intermediate- and high-risk PCa, respectively. Spironolactone use had no effect on the risk of metastatic PCa diagnosis. [14]

It was found that the risk of prostate cancer decreased directly with dose, implying that an increase in dose reduced the chance of prostate cancer. In contrast, the link between spironolactone exposure and prostate cancer in groups at low and high risk for advanced/metastatic illness was not statistically significant. Between symptomatic and asymptomatic patients, the risk of prostate cancer was reduced equally. Prostate cancer risk is decreased by spirolactone by 17%. [15]

Conclusion

Prostate cancer cells multiply more quickly when testosterone is present, however after 48 hours of incubation, high testosterone concentrations induce a (highly significant) ($p<0.001$) decline in cell viability. Spironolactone has an antiproliferative effect on the LNCaP cell line due to its antiandrogenic effects.

Reference

Michel Bolla and Hendrik van Poppel, management of prostate cancer 2017. pp.1

Daniyal, Zamir Ali, Muhammad Akram, HM Asif , Sabria sultana. et al. (2014) 'MINI-REVIEW Epidemiology , Etiology , Diagnosis and Treatment of Prostate Cancer', 15, pp. 9575–9578.

Maurie Markman, MD, President, Medicine & Science at CTCA., cancer treatment center of America, updated on September 21, 2021.

Corvol P, Michaud A, Menard J, Freifeld M, and Mahoudeau J. Endocrinology. 1975 Jul;97(1):52-8. doi: 10.1210/endo-97-1-52.

Claire Gillespie, sciencing. How Does Sonication Work? 2018.

Bosland, M. C. (2014) 'Testosterone treatment is a potent tumor promoter for the rat prostate', Endocrinology, 155(12), pp. 4629–4633.

Morrissey , Ailin Zhang , Jared Lucus , Brett March , AL vin .M . et al. (2020) 'Testosterone accumulation in prostate cancer cells is enhanced by facilitated diffusion ', 79(13), pp. 1530–1542.

Youyuan Xu, Shao-Yong Chen, Kenneth N Ross and Steven P Balk, National Library of Medicine pubmed , Androgens induce prostate cancer cell proliferation through mammalian target of rapamycin activation and post-transcriptional increases in cyclin D proteins, 2006, 1;66(15):7783-92. doi: 10.1158/0008-5472.CAN-05-4472.

Morgentaler, A. and Traish, A. M. (2009) 'Shifting the Paradigm of Testosterone and Prostate Cancer: The Saturation Model and the Limits of Androgen-Dependent Growth', European Urology, 55(2), pp. 310–321.

Vasilia angnostopoulou , Losif Pediaditakis , Saad AL Kahtani , Saud A.Alarafi, Eva mari. et al. (2013) 'Differential effects of dehydroepiandrosterone and testosterone in prostate and colon cancer cell apoptosis: The role of nerve growth factor (NGF) receptors', Endocrinology, 154(7), pp. 2446–2456.

Tiziana Siciliano, Ulrich Sommer, Alicia-Marie K. Beier, Matthias B. Stope, Angelika Borkowetz , Christian Thomas and Holger H. H. Erb, Current Issues in Molecular Biology , The Androgen Hormone-Induced Increase in Androgen Receptor Protein Expression Is Caused by the Autoinduction of the Androgen Receptor Translational Activity, 2022, 44, 597–608. https://doi.org/10.3390/cimb44020041.

Isla S. Mackenzie,Steven V. Morant,Li Wei,Alastair M. Thompson and Thomas M. MacDonald . British Pharmacological society Journal. Spironolactone use and risk of incident cancers: a retrospective, matched cohort study, Citations: 27, 2016.

P C Walsh and P K Siiteri, National Library of medicine , Suppression of plasma androgens by spironolactone in castrated men with carcinoma of the prostate, 114(2):254-6. doi: 10.1016/s0022-5347(17)67001-0, 1975.

Jody A. Charnow Spironolactone Use Associated With Reduced Prostate Cancer Risk, 2020.

Samhita Vitta Spironolactone - A Hypertension Diuretic Drug Reduces Prostate Cancer Risk, 2020.

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Comparative Study of Testosterone, Spironolactone, Docetaxel and Their Combination Effect on Prostate Cancer Cell Line LNCaP

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Abstract

Background: Prostate cancer is the (most frequent) type of cancer among males. Androgenic hormones have a big role in prostate cancer, which is a kind of glandular malignant neoplasia. Testosterone hormone play important role in the cancer cell propagation) and invasion into (surrounding tissue) by cell migration, which is the first stage in tumor metastasis.

Aim of the study: The aim of the study is to determine the effects of testosterone, spironolactone, docetaxel, combination of either testosterone plus spironolactone or testosterone plus spironolactone and docetaxel on the viability percentage of (LNCaP) cell line.

Materials and Methods: This study used the LNCaP (androgen-sensitive human prostate cancer) cell line, which was treated with various concentrations of testosterone, spironolactone, docetaxel, and a combination of testosterone plus spironolactone and testosterone plus spironolactone and docetaxel. After 48 hours of incubation, the MTT test was used to analyze their influence on the viability (proliferative or antiproliferative effect) of the LNCaP cell line.

Results: At low concentrations, testosterone increased the viability of LNCaP cells significantly ($p>0.05$), whereas at high concentrations, it caused a (highly significant) ($p <0.001$) decrease in viability. Spironolactone treatment increased the viability of the LNCaP cell line significantly ($p>0.05$), but it decreased the viability of the LNCaP cell line at high concentrations. In a dose-independent way, docetaxel-treated cells demonstrated a highly significant ($p<0.001$) decrease in viability. The viability of (LNCaP) cell line treated with the combination of testosterone plus spironolactone was high significantly ($P<0.001$) decreased, and in the cells treated with the combination of testosterone pus spironolactone and docetaxel the viability was significantly ($p < 0.05$) decreased by low concentration and high significantly ($p < 0.001$) decreased at 250+250+250 and 500+500+500 µg/ml.

Conclusion: On the LNCaP cell line, high testosterone and spironolactone concentrations had an antiproliferative effect.

Key Words: Testosterone, Spironolactone, LNCaP cell line, prostate cancer

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Introduction

The second (most frequent) cancer in men and the (fifth most common) cause of death globally is prostate cancer. Incidence of (prostate cancer) and fatality rates are directly linked to age, with older men having the greatest incidence (more than 65 years old). [1] According to (GLOBOCAN) estimates, 1,276,106 new cases will be registered worldwide in 2020, with 449,761 in Europe and 64,955 in France. Meanwhile, 358,989 men died of cancer (worldwide, with 107,315 in Europe and 9002 in France. [2] Because androgens play a role in a variety of illnesses, androgen receptor signaling is a critical factor in pathological states. The expression of genes involved in sexual development, prostate cell proliferation and survival, and, to some extent, cancer progression is regulated by the androgen receptor, a transcription factor that is activated by the testosterone metabolite 5-dihydrotestosterone.

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[3] Spironolactone is a nonselective aldosterone receptor antagonist. Its antiandrogenic action lowers testosterone, (androstenedione, and dehydroepiandrosterone) levels in (castrated males) with prostate cancer, making it a potentially effective medicine in the treatment of prostate cancer. Its antiandrogenic properties are supported by a report of a clinical and (prostate specific antigen (PSA) response) in a man with prostate cancer treated with spironolactone. [4] Spironolactone prevents cancer stem cells from growing by inhibiting the DNA damage response. Spironolactone activates the ataxia-telangiectasia mutant checkpoint pathway ATM-Chk2-mediated checkpoint pathway in (colon cancer) cell lines, upregulating the expression of a set of major histocompatibility complex class I-like molecules known as the Natural Killer Group 2D (NKG2D) ligands. [5] Docetaxel (Taxotere®), a cytotoxic taxane with antineoplastic action against a variety of cancer cells, has shown synergistic effect with a number of other anticancer drugs. It is a cytotoxic antimicrotubular drug that inhibits normal cell division by promoting and stabilizing microtubule assembly while also preventing microtubule depolymerization. [6] For metastatic prostate cancer, docetaxel is the first-line chemotherapeutic drug. The emergence of resistance, on the other hand, reduces its efficacy and decreases the survival advantage. [7]

Materials And Methods

The (androgen-dependent) human prostate (adenocarcinoma) cells derived from lymph node metastases that make up the LNCaP cell line were cultured in RPMI-1640 media with the addition of (penicillin) (100 U/ml), (streptomycin) (100 g/ml), and 5% (fetal bovine serum) at (37°C) in 5% CO2. (LNCaP cells) were seeded in tissue culture 96-well plates at a density of 5*105 cells/ml before 24 hours of the treatment with either testosterone (Testopel) 100mg/1ml, spironolactone(Aldacton) 25mg, docetaxel (Taxotere®) 120mg/6ml, combination of testosterone plus spironolactone or testosterone pus spironolactone and docetaxel. Distilled water 5ml was used to dissolve spironolactone and placed in sonicator at 37 C0. Sonication accelerates the dissolution of a solid into a liquid by agitating particles in a solution with sound waves [8] , which is then diluted with complete growth media to obtain final concentrations of (1000, 500, 250, 125, 62.5, 31.25 µg/ml) for spironolactone, also similar concentrations were prepared for both testosterone and docetaxel. The combination of spironolactone plus testosterone was prepared by adding equal concentrations of each agent (500+500, 250+250, 125+125, 62.5+62.5, 31.25+31.25, 15.625+15.625 µg/ml), similarly the combination contain spironolactone plus testosterone and docetaxel was prepared by adding equal concentration of each agent (500+500+500, 250+250+250, 125+125+125, 62.5+62.5+62.5, 31.25+31.25+31.25, 15.625+15.625+15.625 µg /ml). Following that, 200 µl of each concentration was poured into each well and left to incubate for another 48 hours. After the 48-hour exposure period, the wells were cleaned with 200 µl of (sterile PBS). The MTT assay was used to investigate the effects of testosterone, spironolactone, docetaxel, and combinations of testosterone plus spironolactone or testosterone plus spironolactone and docetaxel on the growth of the (LNCaP) cell line. The (MTT assay) determines how quickly a (tetrazolium salt) is converted into a (formazan product) in the cell (purple color). The opacity of the purple color is (directly proportional) to the number of live cells; this may be evaluated using (spectrophotometry) and gives a relative estimate of cell viability. Three replicates for each concentration was considered. Microsoft Office Excel 2010 was used to collect and analyze all of the data. A one-way Anova test was used to examine the differences between each treated group and the (control group). P-values (≤0.05) and (≤0.001) were considered statistically (significant) and (highly significant) respectively.

Results

Comparing to the control group (untreated cells), result showed that after 48 hours of incubation testosterone caused a significant increase ($p < 0.05$) in the viability of LNCaP cell line at the concentrations 125 and 62.5 µg/ml, while it caused a significant ($p < 0.05$) and a (highly significant) ($p < 0.001$) decrease in the viability of these cells at the concentration 250 µg/ml and at the concentrations 500 and 1000 µg/ml respectively as shown in (figure. 1).

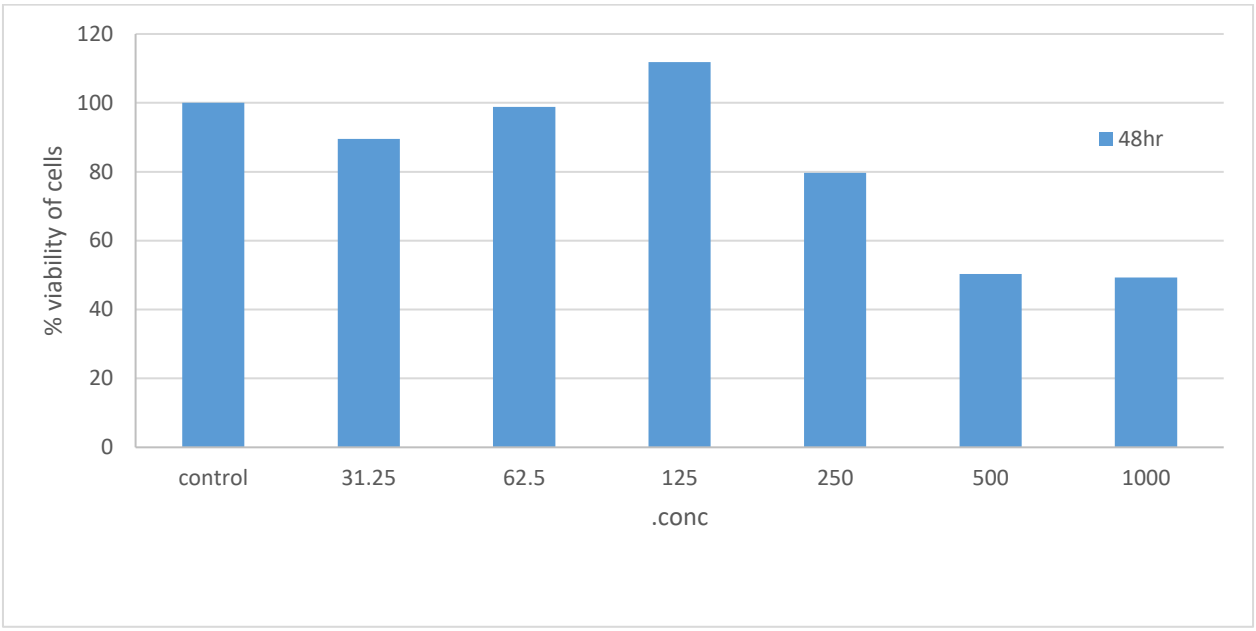

Figure 1: Effect of (Testosterone) on the (Viability) Percentage of LNCaP cell line after 48 hours of incubation

Comparing to the control group (untreated cells), result showed that after 48 hours of incubation spironolactone caused (a significant) ($p< 0.05$) increase in the viability of LNCaP cell line at the cancentration 62.5 µg/ml. While at the concentrations 31.25, 125 and 1000 µg/ml it caused significant decrease in the viability of these cells. Differences between the control group and 250 and 500 µg/ml treated group was insignificant ($p>0.05$) as shown in (figure. 2).

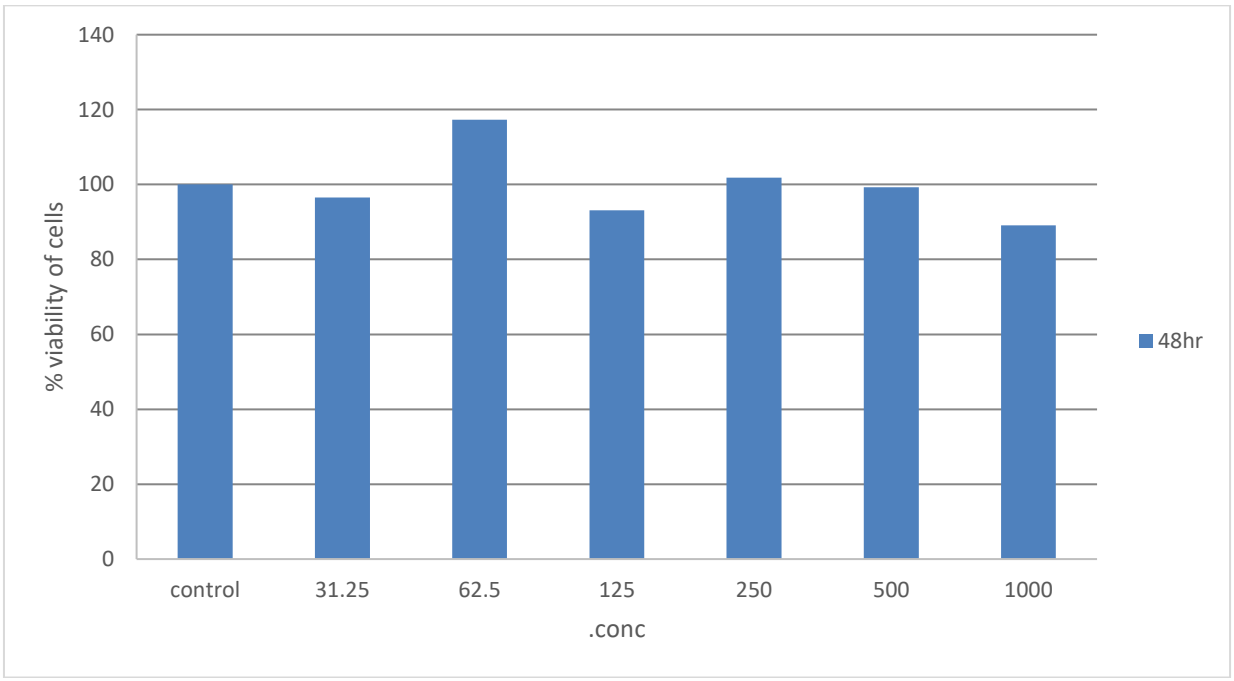

Figure 2: Effect of Spironolactone on the Viability Percentage of LNCaP Cell line after 48 hours of incubation.

Comparing to the control group (untreated cells), result showed that after 48 hours of incubation docetaxel caused a highly significant ($p < 0.001$) decrease in the viability of LNCaP cell line at (all concentrations) as shown in (figure 3)

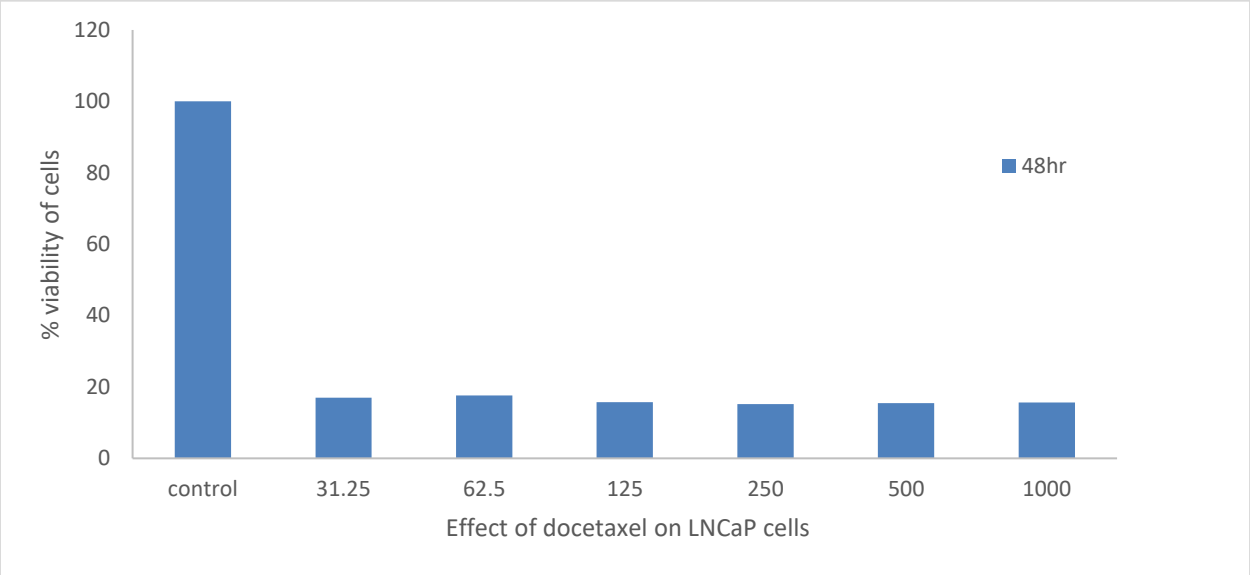

Figure 3: Effect of Docetaxel on the Viability Percentage of LNCaP Cell line after 48 hours of incubation.

Comparing to the control group (untreated cells), result showed that after 48 hours of incubation the combination of spironolactone plus testosterone caused significant (P<0.05) decrease in cell viability at concentration 31.25+31.25 µg/ml while it caused insignificant (p>0.05) in the viability of these cells at all other concentrations as shown in (figure. 4).

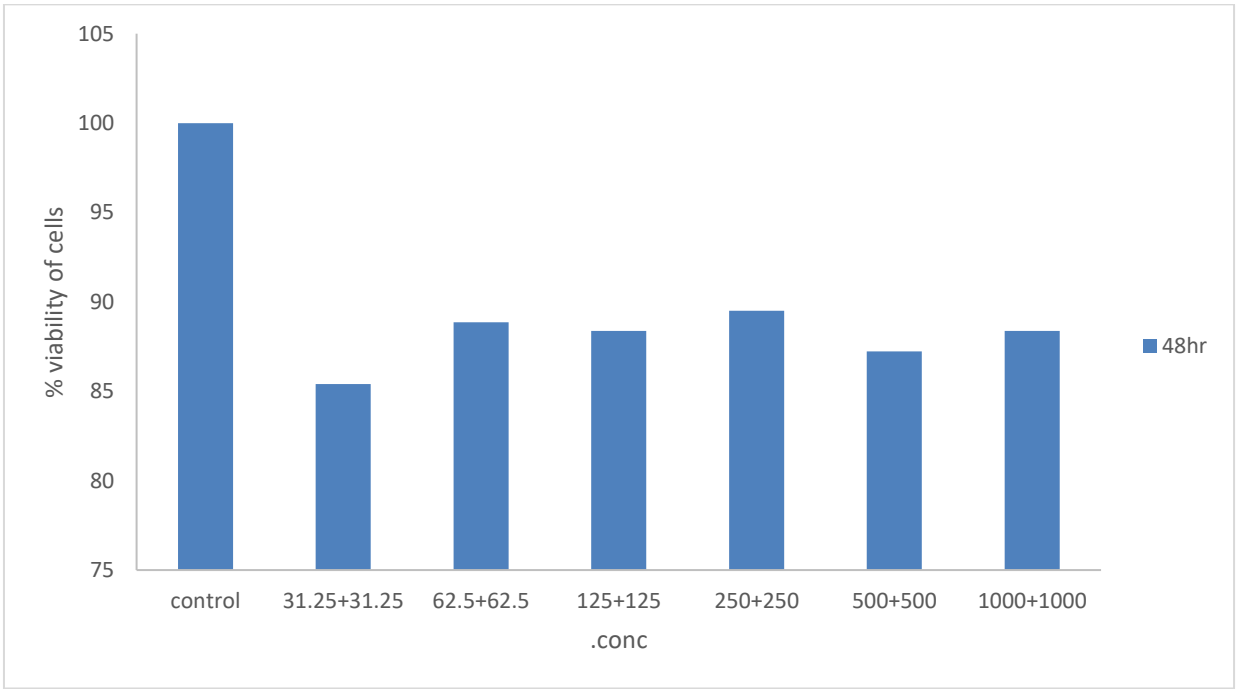

Figure 4: Effect of spironolactone plus testosterone combination on the viability percentage of LNCaP cell line after 48 hours of incubation.

Comparing to the control group (untreated cells), result showed that after 48 hours of incubation the viability of LNCaP cell line was significantly (P<0.05) decreased after treatment with the combination containing 62.5 µg/ml of each component and that containing 125 µg/ml of each component. Difference between the control group and 15.625 µg/ml and 31.25 µg/ml treated groups was insignificant

(p>0.05). Also the combination containing 250 µg/ml and that containing 500 µg/ml of each component decreased the viability of LNCaP cell line high significantly (p<0.001). The combination containing 500 µg/ml of each component showed the maximum cytotoxic effect against the LNCaP cell line (figure 5).

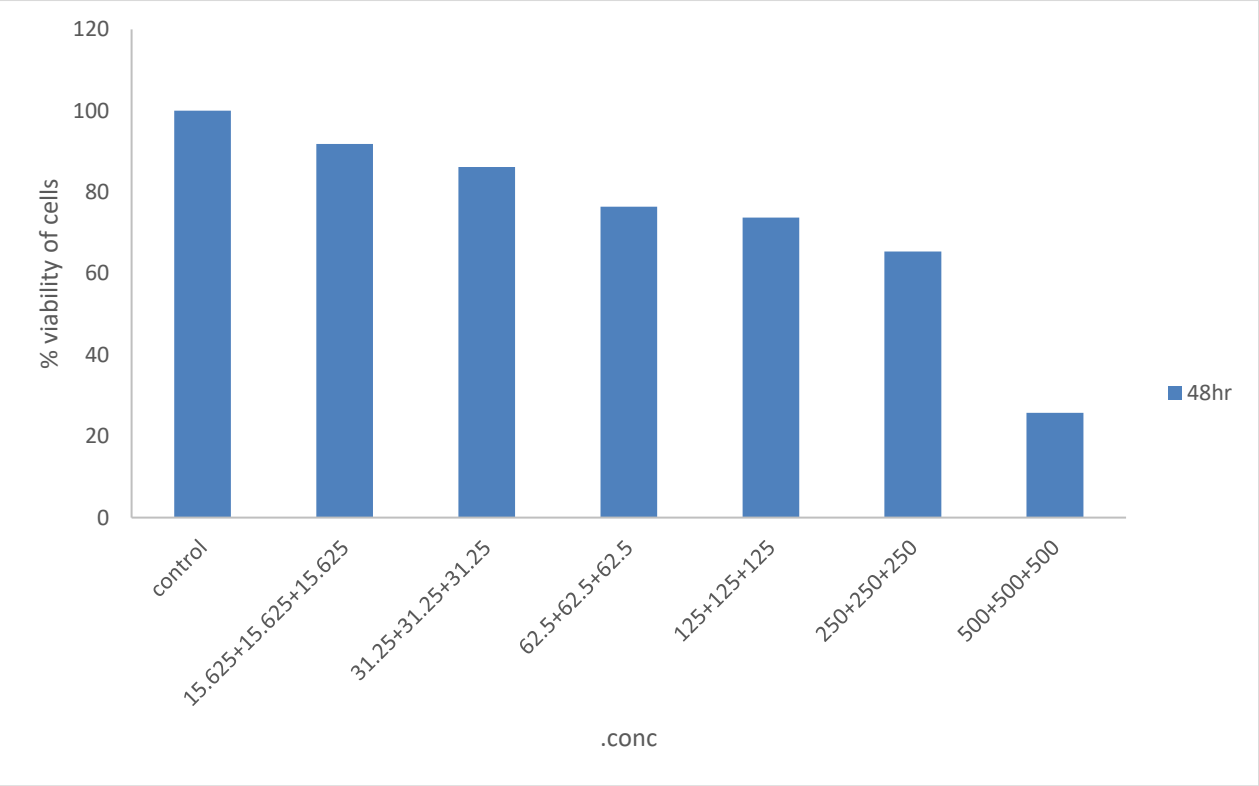

Figure 5: Effect of spironolactone plus testosterone and docetaxel combination on the viability percentage of LNCaP cell line after 48 hours of incubation.

Discussion

Regarding the role of testosterone in promoting or surpassing prostate cancer, evidences are inconsistent. Testosterone is not a tumor-promoting factor for prostate cancer, but it is possible that it caused prostate epithelial cells to develop hypersensitivity to androgens, giving them a selective growth advantage that led to a few of those cells progressing to malignancy (Bosland, 2014). A study by Anagnostopoulou et al., (2013) stated that in animal models, testosterone has been shown to inhibit cancer cell proliferation by counteracting the proliferative effects of endogenous hormones like DHEA. [9]

Because very high androgen levels inhibit the proliferation of prostate cancer cells, this effect could be used in prostate cancer treatment. However, these pathways have largely remained undiscovered at the molecular and cellular levels. As a result, the purpose of this study is to see how different testosterone concentrations affect the viability of (prostate cancer) cells. High testosterone has an inhibitory effect on cell viability in the LNCaP cell line due to androgenic receptor (AR) downstream signaling or non-genomic AR activity. Furthermore, hormonal activation of the androgen receptor causes the receptor to self-stabilize, resulting in an increase in AR activity. As a result, in clinical practice, a therapeutic reduction in androgen levels is a clinical target that would result in a drop in AR activity and, as a result, a decline in AR-driven prostate cancer progression. [10]

The viability of the (LNCaP) cell line increased after treatment with a low dosage of spironolactone, but decreased significantly when the concentration was increased to 1000 µg/ml. These results agree with Isla, (2016) who found that taking spironolactone was linked to a decreased risk of prostate cancer in a retrospective, matched cohort study. [11] Also, this result agrees with Walsh, (1975) who found that spironolactone can suppress the level of plasma

androgens by spironolactone. These findings could be attributed to the effect of spironolactone as an inhibitor of androgen synthesis, as found in a Walsh study in which spironolactone was given to castrated men with metastatic prostate cancer. This study found that spironolactone decreased the amounts of the androgens produced by the adrenal glands, including testosterone, androstenedione, and dehydroepiandrosterone. [12]

The decreased in the viability of the (LNCaP) cell line after treatment with all concentrations of docetaxel agrees with Yang et al., (2019) study that docetaxel suppresses the development of cultured prostate cancer cells in a dose-independent way. These findings are connected to the function of docetaxel as a microtubule inhibitor, which prevents microtubule disintegration by binding to -tubulin. [13] As a result, by arresting cells in the (G2/M phase) of the cell cycle, docetaxel causes cell death, which peaked at 24 and 48 hours. Docetaxel inhibited PI3K/Akt activation, lowered Bcl-2 levels, and enhanced caspase-3/9 activation, and it had a higher (inhibitory effect) on (AR-dependent LNCaP cells) than on AR-independent PC3 cells on growth suppression and apoptosis in prostate cancer cells. [14]

After 48 hours of incubation, the testosterone and it combination with spironolactone was significantly ($P < 0.05$) reduced the viability of the LNCaP cell line. These findings are agree with Jeffrey (2015), who found that despite historical evidence that suggests testosterone is generally hazardous for men with active malignancies, testosterone therapy to (castrate-resistant patients) may help in restoring (hormone sensitivity) and hence aid in transforming bad tumours into a (less aggressive phenotype). Prostate cancer cell development is suppressed by high testosterone concentrations, whereas cell growth is stimulated by low testosterone doses. Therefore, the researcher believe it is dangerous to use excessive doses of testosterone in men who are hormone resistant. It may be particularly wrong to give testosterone to males who have significant prostate tumors. [15]

The viability of the LNCaP cell line was significant ($P < 0.001$) reduced in a time-dependent and concentration dependent fashion after treatment with the mixture containing 250 µg/ml and that containing 500 µg/ml of each of testosterone, spironolactone and docetaxel after 48 hours of incubation. These findings are agree with Paula et al., 2021 they found that docetaxel-induced decrease of free testosterone blood levels in patients with metastatic prostate cancer plays a predictive significance. Docetaxel affects androgen receptor signaling, whereas testosterone reduces docetaxel absorption by cells and prevents microtubule stability. As a result, it was suggested that testosterone levels be reduced while receiving docetaxel chemotherapy.Although blood androgens (testosterone, androstenedione, and DHEA) decrease following docetaxel treatment, its effect on lowering testosterone levels is less evident. The majority of testosterone 6β- and 16β-hydroxylation is catalyzed by CYP3A4, which is also primarily responsible for the metabolism of docetaxel. Docetaxel has been demonstrated to induce CYP3A4. Through inactivation through 6β- and 16β-hydroxylation, CYP3A4 upregulation can reduce testosterone levels. [16]

Conclusion

All high concentrations of testosterone have an antiproliferative rather than proliferative effect on the LNCaP cell line. Spirolactone has an antiproliferative impact.

Reference

Rawla, Prashanth, Epidemiology of Prostate Cancer, World J Oncol. 2019;10(2):63-89.

Wendy Bijoux, Emilie Cordina-Duverger, Soumaya Balbolia, Pierre-Jean Lamy, Xavier Rebillard, Brigitte Tretarre, Sylvie Cenee and Florence Menegaux. Occupation and prostate Cancer risk: results from the epidemiological study of prostate cancer (EPICAP) Journal of Occupational Medicine and Toxicology. 2022; 17(5).

Sebastian Student, Tomasz Hejmo, Aleksandra Poterała-Hejmo, Aleksandra Leśniak and Rafał Bułdakc. Anti-androgen hormonal therapy for cancer and other diseases, European Journal of Pharmacology. 2020; 866 (172783).

Santhanam Sundar and Peter D Dickinson , PMC PubMed Central® Spironolactone, a possible selective androgen receptor modulator, should be used with caution in patients with metastatic carcinoma of the prostate , 2012, doi: 10.1136/bcr.11.2011.5238.

Rajanna A. Novel approach to target cancer stem cells for therapy. Med Hypotheses. 2016;88:83–5.

Katherine A. Lyseng-Williamson and Caroline Fenton , Docetaxel: a review of its use in metastatic breast cancer 2005, 65 (17).

Lu, Xinxing , Yang, Feiya ,Chen, Dexi ,Zhao, Qinxin ,Chen, Dong ,Ping, Hao Xing, Nianzeng , Quercetin reverses docetaxel resistance in prostate cancer via androgen receptor and PI3K/AKT signaling pathways , International Journal of Biological Sciences, 2020, 16(7) .

Claire Gillespie, sciencing. How Does Sonication Work? 2018.

Vasilia angnostopoulou , Losif Pediaditakis , Saad AL Kahtani , Saud A.Alarafi, Eva mari. et al. 'Differential effects of dehydroepiandrosterone and testosterone in prostate and colon cancer cell apoptosis: The role of nerve growth

factor (NGF) receptors', Endocrinology. 2013; 154(7), pp. 2446–2456.

Tiziana Siciliano, Ulrich Sommer, Alicia-Marie K. Beier, Matthias B. Stope, Angelika Borkowetz , Christian Thomas and Holger H. H. Erb, Current Issues in Molecular Biology , The Androgen Hormone-Induced Increase in Androgen Receptor Protein Expression Is Caused by the Autoinduction of the Androgen Receptor Translational Activity. 2022; 44, 597–608.

Isla S. Mackenzie,Steven V. Morant,Li Wei,Alastair M. Thompson and Thomas M. MacDonald, Spironolactone use and risk of incident cancers: a retrospective, matched cohort study, British pharmacological society journals. 2016; https://doi.org/10.1111/bcp.13152Citations: 28.

P C Walsh and P K Siiteri, Suppression of plasma androgens by spironolactone in castrated men with carcinoma of the prostate, National Library of medicine. 1975; 114(2):254-6. doi: 10.1016/s0022-5347(17)67001-0.

Chongyi Yang , Weijie Zhang , Jie Wang, Pengpeng Chen and Jiangjiang Jin, Effect of docetaxel on the regulation of proliferation and apoptosis of human prostate cancer cells, National Library of Medicine. 2019; 19(5):3864-3870. doi: 10.3892/mmr.2019.9998. Epub.

Nehmé , P.Varadarajan , Q Zhang, M Gerhold , X Lin. et al. 'Modulation of docetaxel-induced apoptosis and cell cycle arrest by all-trans retinoic acid in prostate cancer cells', British Journal of Cancer. 2001; 84(11), pp. 1571–1576

Jeffrey Turner and Marina Is testosterone the new therapy for prostate cancer ? prostate cancer research institute 2015, Is. 18 Vol. 4.

Paula Kappler, Michael A. Morgan, Philipp Ivanyi, Stefan J. Brunotte, Arnold Ganser and Christoph W. M. Reuter, Prognostic role of docetaxel-induced suppression of free testosterone serum levels in metastatic prostate cancer patients, Scientific Reports volume 11, Article number: 16457 (2021)

3. Results

3.1. Effect of testosterone on the viability of LNCaP cell line after 24 and 48 hours of incubation:

Results showed that the concentrations 1000,500 and 250 µg/ml of testosterone cause significant ($p<0.05$) increase in the viability of LNCaP cell line in comparison to the control group after 24 hours of incubation. There were no significant differences between the control group and 62.5, 125 µg/ml groups for the same time of incubation.

After 48 hours of incubation results showed that the testosterone concentrations 125 µg/ml cause insignificant ($P<0.05$) increase in the viability of LNCaP cell line, while testosterone concentration 250 µg/ml cause significant ($p<0.05$) decrease also 500 and 1000 µg/ml cause significant ($p<0.001$) decrease in the viability of LNCaP cell line when compared to the control group. There were no significant differences between the control group and 62.5 µg/ml groups for the same time of incubation as shown in (figure 3.1).

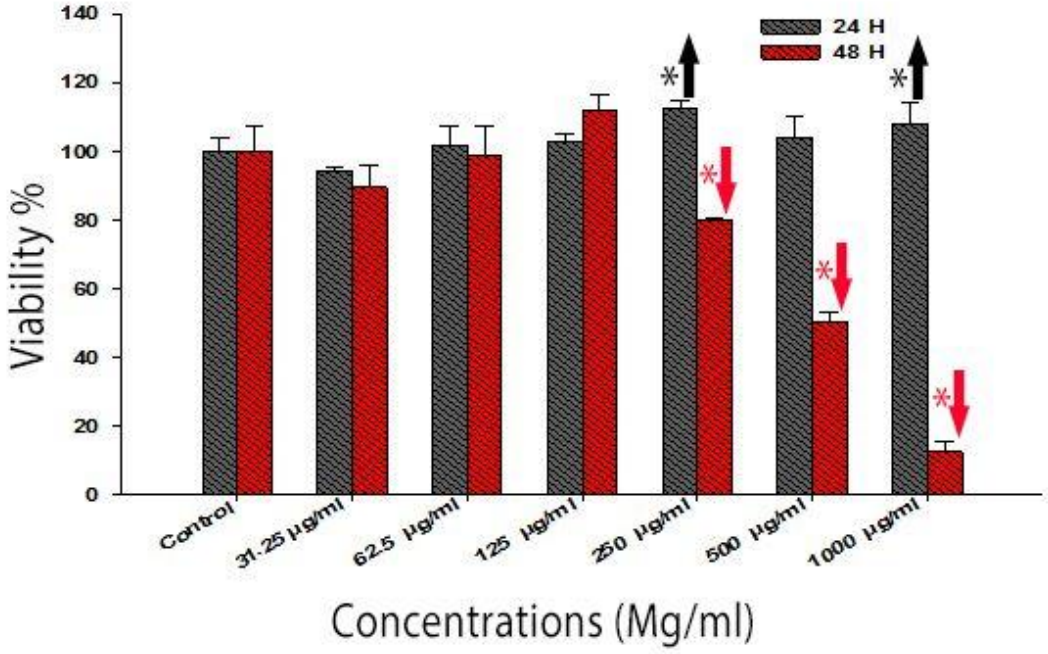

Figure (3.1) Effect of testosterone on the Viability Percentage of LNCaP cell line after 24 and 48 hours of incubation

3.2 Effect of spironolactone on the viability percentage of LNCaP cell line after 24 and 48 hours of incubation.

Results showed that the concentrations 31.25, 62.5 and 125 µg/ml of spironolactone cause insignificant ($p<0.05$) change in the viability of LNCaP cell line where as the concentration 500 and 1000 µg/ml cause significant decrease in the viability of LNCaP cell line in comparison to the control group after 24 hours of incubation.

After 48 hours of incubation results showed that the spironolactone at concentration 62.5 µg/ml causes a significant (P<0.05) increase in cell viability when compared with the control group. While at concentration 1000 µg/ml causes a significant decrease in cells viability. Also the result showed that there were insignificant (p<0.05) decrease in the viability of LNCaP cell line in comparison to control group. There were no significant differences between the control group and 250 and 500 µg/ml groups for the same time of incubation as shown in (figure 3.2).

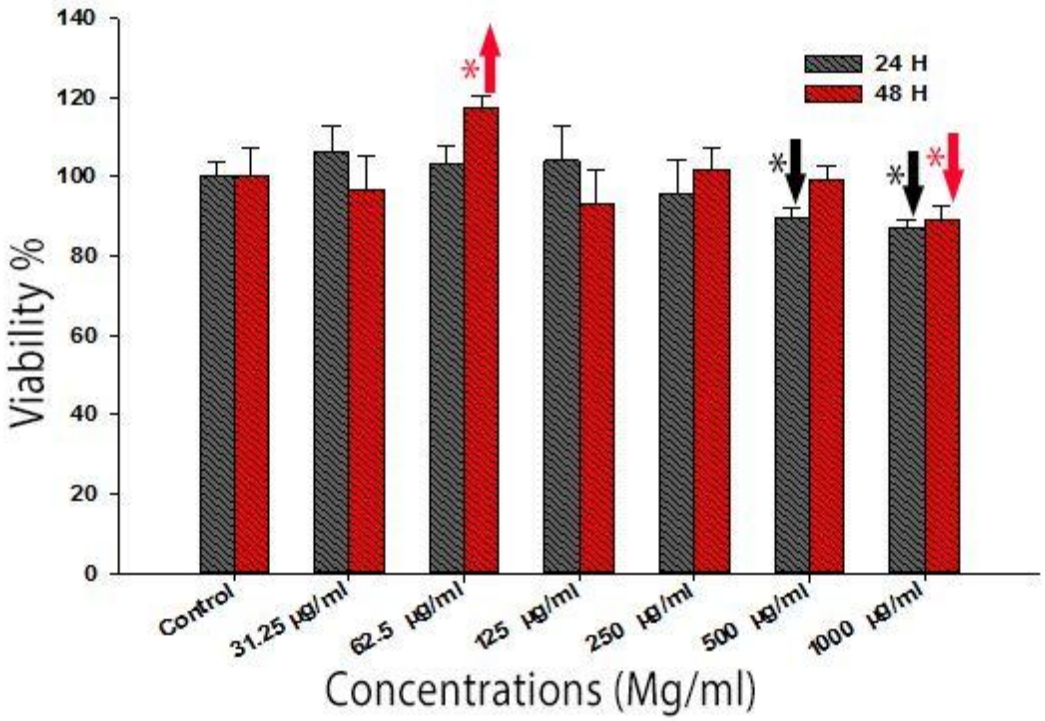

Figure (3.2) Effect of spironolactone on the viability percentage of LNCaP cell line after 24 and 48 hours of incubation.

3.3. Effect of docetaxel on the viability Percentage of LNCaP cell line after 24 and 48 hours of incubation.

In comparison to the control group, result showed that the concentration 250 µg/ml of docetaxel cause significant ($p<0.05$) decrease in the viability of LNCaP cell line and the concentrations 500 and 1000 µg/ml cause highly significant ($p<0.001$) decrease in the viability of LNCaP cell line, while no significant difference ($p<0.05$) were found between the control and the concentrations 31.25, 62.5 and 125 µg/ml after 24 hours of incubation.

Also results showed that after 48 hours of incubation all concentrations of docetaxel cause highly significant ($p<0.001$) decrease in the viability of LNCaP cell line in comparison to the control group.

According to the time of incubation, the results revealed that docetaxel causes a highly significant ($p < 0.001$) decrease in the viability of LNCaP cell line at all concentrations after incubation for 48 hours but it caused significant ($P < 0.05$) decrease in the viability of LNCaP cell line at high concentrations after incubation for 24 hours as shown in (figure 3.3).

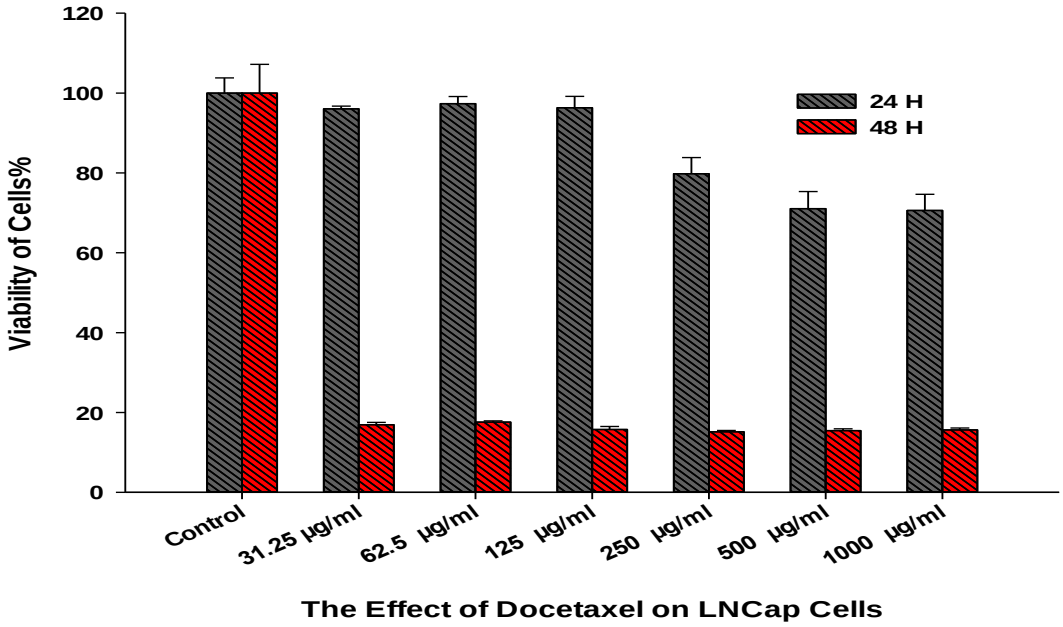

Figure (3.3) Effect of docetaxel on the viability Percentage of LNCaP cell line after 24 and 48 hours of incubation.

3.4 Effect of spironolactone plus testosterone combination on the viability percentage of LNCaP cell line after 24 and 48 hours of incubation.

The results showed there were a highly significant ($P<0.001$) decrease in cell viability at concentrations 31.25, 62.5, 500 and 1000 µg/ml. while at concentration 125 and 250 µg/ml there were a significant ($P<0.05$) increase in cell viability when compared with control group after incubation for 24 hours. While there were significant ($P<0.05$) decrease in cell viability at all concentrations in the same level when compared with the control group after 48 hours of incubation.

The results indicated that the testosterone plus spironolactone combination induces a significant ($P<0.05$) drop in the viability of LNCaP cell line depending on the length of incubation, with a highly

significant (P<0.001) decrease in the viability of LNCaP cell line after 24 hours compared with 48 hours as shown in (Fig 3.4).

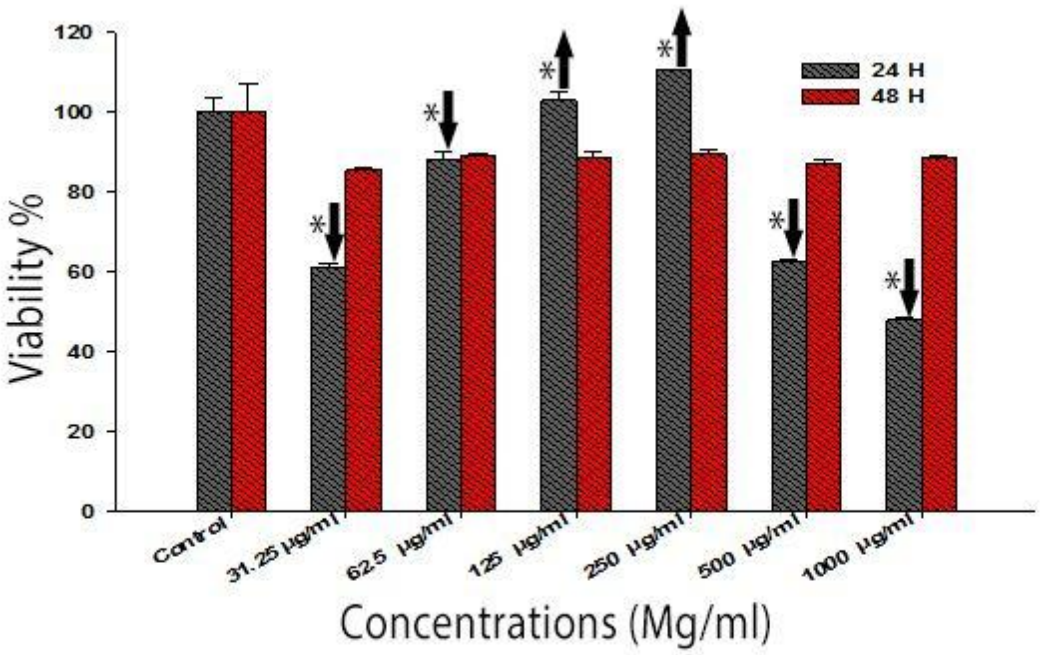

Figure (3.4) Effect of spironolactone plus testosterone combination on the viability percentage of LNCaP cell line after 24 and 48 hours of incubation.

3.5 Comparison the effects of testosterone with spironolactone-testosterone combination on the viability percentage of LNCaP cell line after 24 hours of incubation

Results showed that the concentrations 1000,500 and 250 µg/ml of testosterone cause significant ($p<0.05$) increase in the viability of LNCaP cell line in comparison to the control group after 24 hours of incubation. There were no significant differences between the control group and 62.5, 125 µg/ml groups for the same time of incubation. There were a highly significant ($P<0.001$) decrease in cell viability at concentrations 31.25, 62.5, 500 and 1000 µg/ml of spironolactone-testosterone combination. while at concentration 125 and 250 µg/ml there were a significant ($P<0.05$) increase in cell viability when compared with control group after incubation for 24 hours as shown in (Fig 3.5).

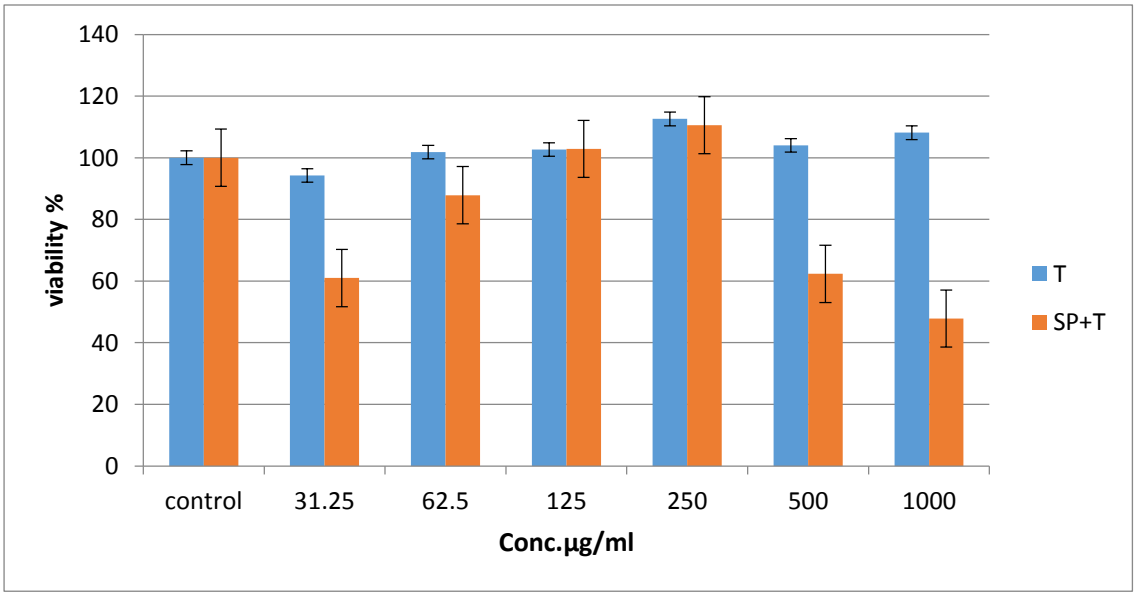

Figure 3.5 Comparison the effects of testosterone with spironolactone-testosterone combination on the viability percentage of LNCaP cell line after 24 hours of incubation

3.6 Comparison the effects of testosterone with spironolactone-testosterone combination on the viability percentage of LNCaP cell line after 48 hours of incubation

After 48 hours of incubation results showed that the testosterone concentrations 125 µg/ml cause insignificant ($P<0.05$) increase in the viability of LNCaP cell line, while testosterone concentration 250 µg/ml cause significant ($p<0.05$) decrease also 500 and 1000 µg/ml cause significant ($p<0.001$) decrease in the viability of LNCaP cell line when compared to the control group. There were no significant differences between the control group and 62.5 µg/ml groups for the same time of incubation. While there were significant ($P<0.05$) decrease in cell viability at all concentrations in the same level when compared with the control group after 48 hours of incubation as shown in (Fig 3.6).

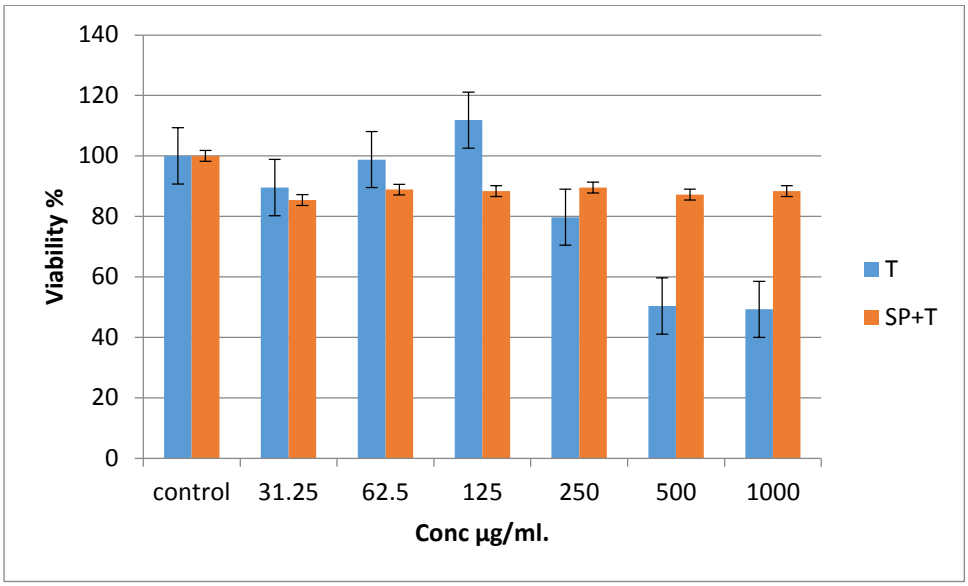

Figure 3.6 Comparison the effects of testosterone with spironolactone-testosterone combination on the viability percentage of LNCaP cell line after 48 hours of incubation

3.7 Comparison the effect of docetaxel with docetaxel plus 500µg/ml spironolactone combination on the viability percentage of LNCaP cell line after 24 hours of incubation

In comparison to the control group, result showed that the concentration 250 µg/ml of docetaxel cause significant ($p<0.05$) decrease in the viability of LNCaP cell line and the concentrations 500 and 1000 µg/ml cause highly significant ($p<0.001$) decrease in the viability of LNCaP cell line, while no significant difference ($p<0.05$) were found between the control and the concentrations 31.25, 62.5 and 125 µg/ml after 24 hours of incubation.

Also the results showed that the concentration 500+15.625 µg/ml of docetaxel-spironolactone combination cause significant (P<0.05) increase in the viability of LNCaP cell line, while the concentration 500+31.25 µg/ml cause insignificant (P<0.05) decrease in the viability of LNCaP cell line. There were significant (P<0.05) decrease in the viability of LNCaP cell line at concentrations 500+62.5, 500+125 and 500+250 µg/ml and highly significant (p<0.001) decrease in the viability of LNCaP cell line at concentration 500+500 µg/ml when compared to control group after 24 hours of incubation as shown in (Fig 3.7).

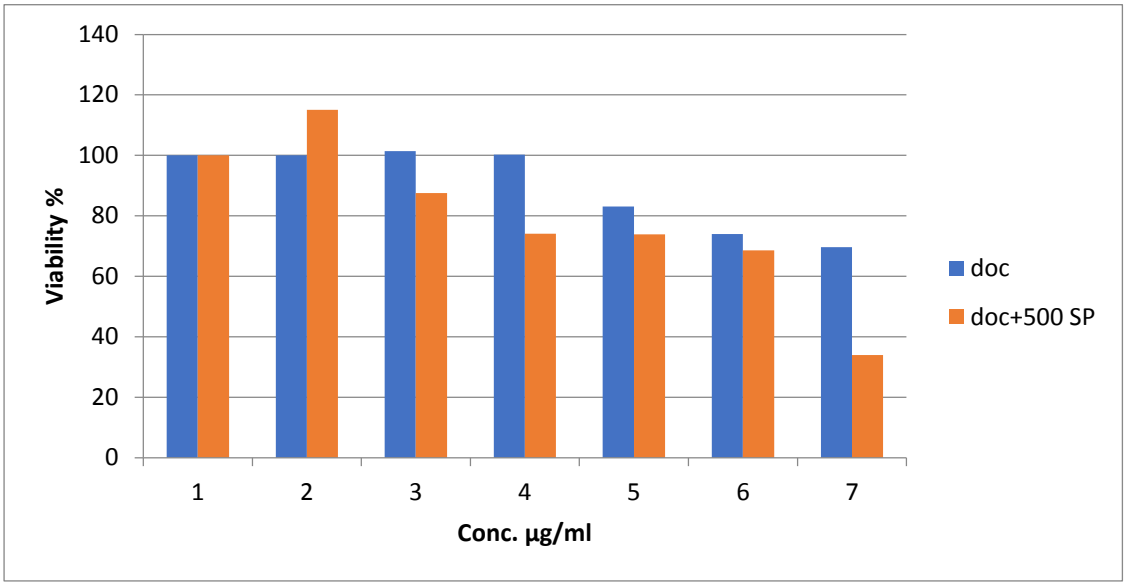

Figure 3.7 Comparison the effect of docetaxel with docetaxel plus 500µg/ml spironolactone combination on the viability percentage of LNCaP cell line after 24 hours of incubation

3.8 Comparison the effect of docetaxel with docetaxel plus 500µg/ml spironolactone combination on the viability percentage of LNCaP cell line after 48 hours of incubation

Results showed that after 48 hours of incubation all concentrations of docetaxel cause highly significant ($p<0.001$) decrease in the viability of LNCaP cell line in comparison to the control group.

Also the result showed that after 48 hours of incubation all combination of docetaxel plus 500 µg/ml spironolactone cause highly significant ($P<0.001$) decrease in the viability of LNCaP cell line in comparison to the control group as shown in (Fig 3.8).

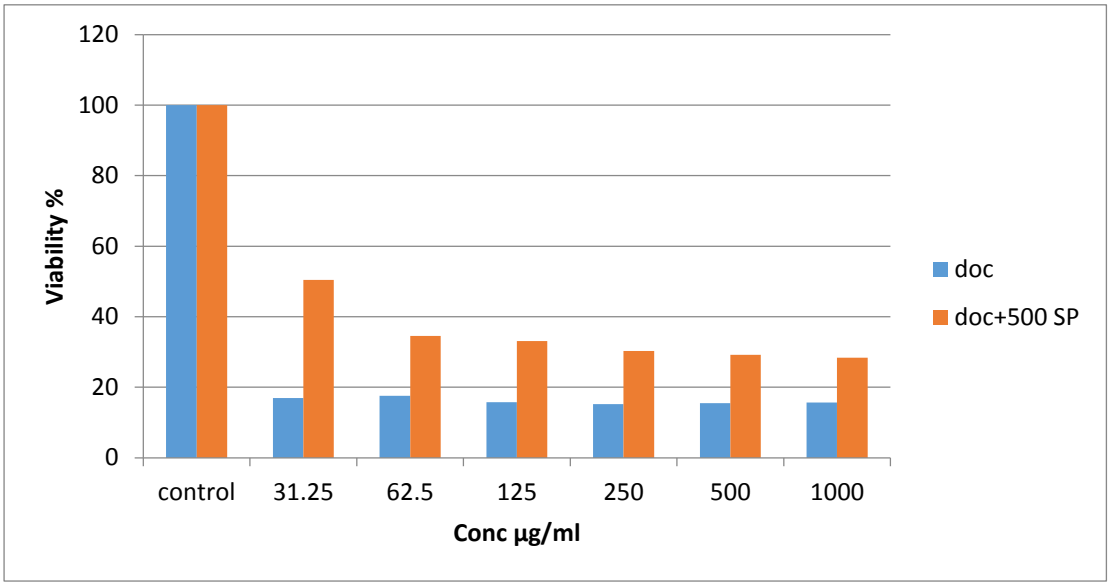

Figure 3.8 Comparison the effect of docetaxel with docetaxel plus 500µg/ml spironolactone combination on the viability percentage of LNCaP cell line after 48 hours of incubation

3.9 Effect of spironolactone different concentrations combined to 500 µg/ml docetaxel on the viability of LNCaP cell line after 24 and 48 hours of incubation:

Results showed that the combination of docetaxel (500µg/ml) plus spironolactone (different concentrations) 500+125, 500+250 and 500+500 µg/ml caused significant ($P< 0.05$) increase in the viability of LNCaP cell line, while concentrations 500+15.625, 500+31.25 and 500+62.5 µg/ml significant differences in the viability of LNCaP cell line when compared to control group were not found ($p<0.05$) after 24 hours of incubation.

Results also showed that after 48 hours of incubation all combination of spironolactone plus 500 µg/ml docetaxel cause highly significant ($p<0.001$) decrease in the viability of LNCaP cell line in comparison to the control group.

According to the time of incubation the results showed there were a significant ($P > 0.05$) increase in cell viability after incubation for 24 hours when compared with incubation for 48 hours that causes a highly significant ($p < 0.001$) decrease in cells viability at all concentrations as shown in (figure 3.9).

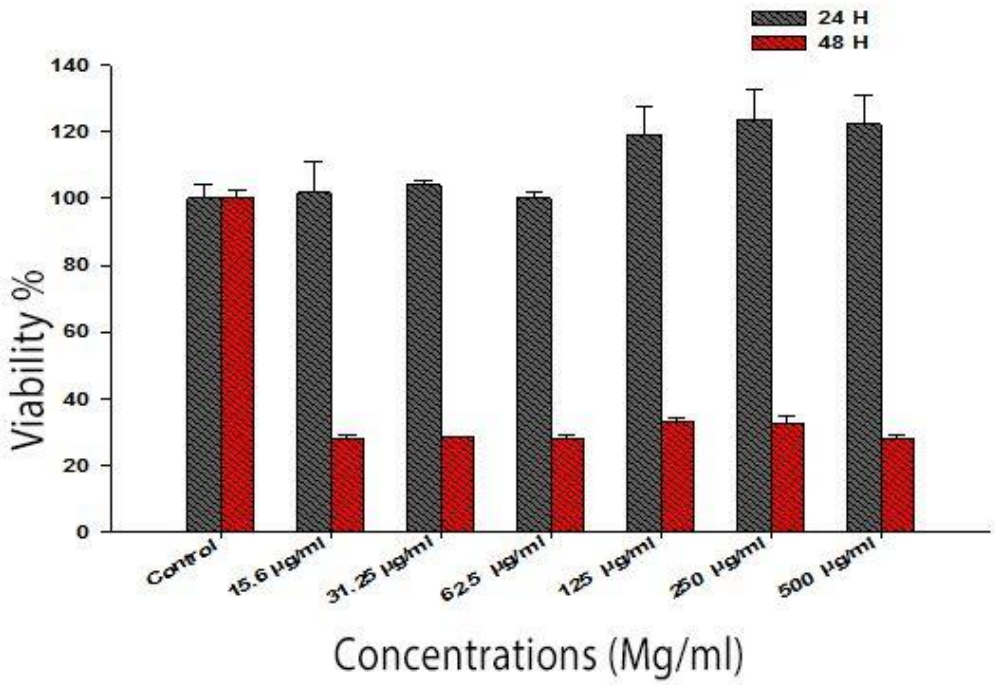

Figure (3.9) Effect of spironolactone different concentrations combined to 500 µg/ml docetaxel on the viability percentage of LNCaP cell line after 24 and 48 hours of incubation

3.10 Effect of docetaxel different concentrations combined to 500µg/ml spironolactone on the viability of LNCaP cell line after 24 and 48 hours of incubation:

Results showed that the concentration 500+15.625 µg/ml cause significant ($P<0.05$) increase in the viability of LNCaP cell line, while the concentration 500+31.25 µg/ml cause insignificant ($P>0.05$) decrease in the viability of LNCaP cell line. Also the results showed significant ($P<0.05$) decrease in the viability of LNCaP cell line at concentrations 500+62.5, 500+125 and 500+250 µg/ml and highly significant ($p<0.001$) decrease in the viability of LNCaP cell line at concentration 500+500 µg/ml when compared to control group after 24 hours of incubation.

Results also showed that after 48 hours of incubation all combination of docetaxel plus 500 µg/ml spironolactone cause highly significant (P<0.001) decrease in the viability of LNCaP cell line in comparison to the control group.

According to the time of incubation the results showed there were a significant (P< 0.05) increase in cell viability at low concentration of docetaxel and highly significant (p<0.001) decrease in cell viability at high concentrations after incubation for 24 hours when compared with incubation for 48 hours that causes a highly significant (p < 0.001) decrease in cells viability at all concentrations as shown in (figure 3.10).

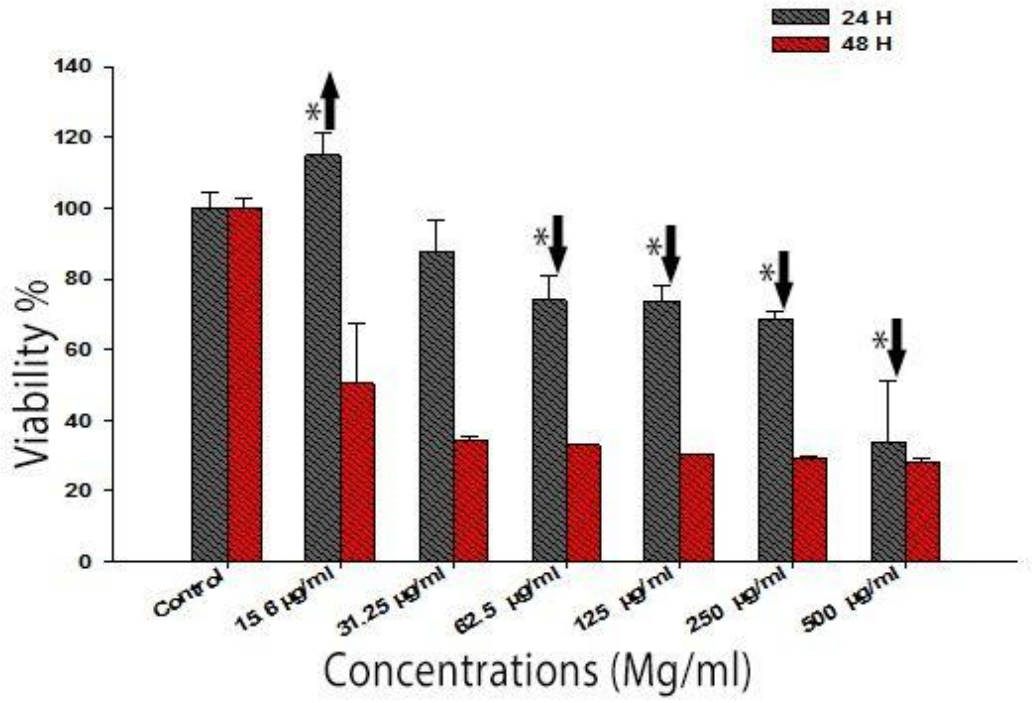

Figure (3.10) Effect of docetaxel different concentrations combined to 500µg/ml spironolactone on the viability of LNCaP cell line after 24 and 48 hours of incubation

3.11 Effect of spironolactone plus testosterone and docetaxel combination on the viability percentage of LNCaP cell line after 48 hours of incubation.

Comparing to the control group (untreated cells), result showed that after 48 hours of incubation the viability of LNCaP cell line was high significantly (P<0.001) decreased after treatment with the combination containing 250 and 500 µg/ml of each component. There was non-significant difference between the control group and 15.625 µg/ml and 31.25 µg/ml treated groups. The combination containing 250 µg/ml and that containing 500 µg/ml of each component decreased the viability of LNCaP cell line high significantly (p<0.001). The combination containing 500 µg/ml of each component showed the maximum cytotoxic effect against the LNCaP cell line (Fig 3.11).

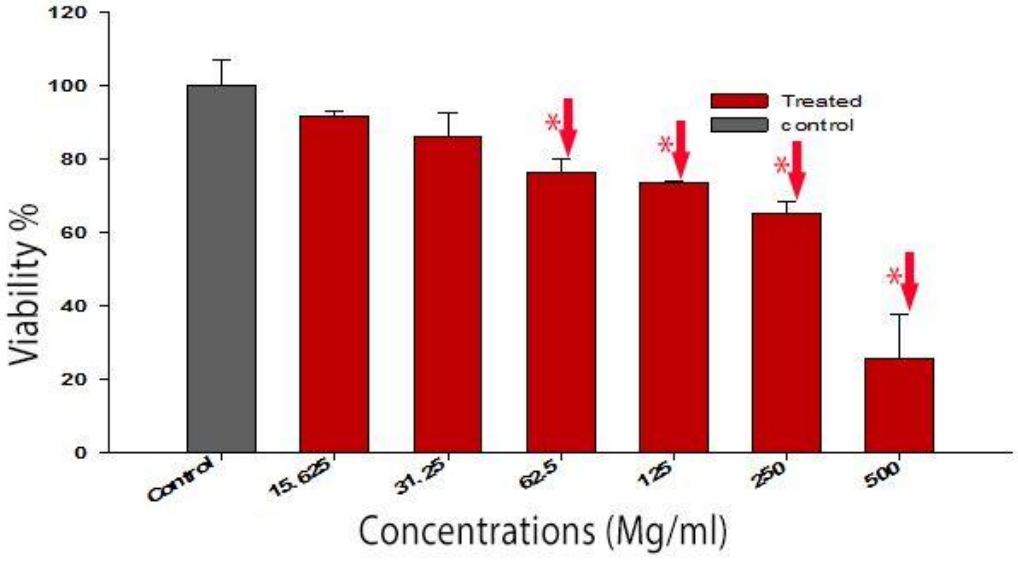

Figure (3.11) Cell viability Percentage of LNCaP cell line of docetaxel in combination with spironolactone and testosterone after incubation for 48 hours by MTT assay

4. Discussion

In men, prostate cancer is one of the commonest type of cancer. In the early stage of prostate cancer different treatment can be used including androgen deprivation therapy, radical prostatectomy, external-beam radiation, and prostate brachytherapy.

For patients who develop metastatic illness, the two main therapeutic modalities are taxanes and androgen deprivation therapy. For castrate-resistant metastatic prostate cancer, taxanes such as paclitaxel, docetaxel, and a combination of docetaxel and platinum are routinely utilized chemotherapy . New medications, such as androgen receptor inhibitors like abiraterone acetate and enzalutamide, have been approved for the treatment of metastatic castration-resistant prostate cancer

4.1. Effect of testosterone on the viability of prostate cancer cell line.

Regarding the role of testosterone in promoting or suppressing prostate cancer, evidences are inconsistent. Testosterone is not a tumor-promoting factor for prostate cancer, but it is possible that it caused prostate epithelial cells to develop hypersensitivity to androgens, that can result in a selective growth advantage thus a few of those cells progressing to malignancy (Bosland, 2014).

Through ligand-mediated activation of the androgen receptor (AR), testosterone increases the proliferation of LNCaP cells, which drives prostate cancer progression (Morrissey *et al.,* 2020). In the prostate, muscle, bone, and adipose tissue, the (AR) plays a critical role. In the prostate tissue the dysregulation in the activity of AR is a driving force for the initiation and progression of (PCa).

Androgens stimulate LNCaP proliferation through PI3K/Akt-independent activation of mammalian Target of Rapamycin (mTOR) and consequent post-transcriptional increases in cyclin D protein production, according to the (AR) (Xu *et al.*, 2006).

This effect profile is agree with Morgentaler and Traish (2009) whom documented that cell proliferation increased gradually with rising testosterone concentrations, then plateaued with no further response despite logarithmically increase in the concentration of testosterone.

Anagnostopoulou *et al.* (2013) study which is an animal model study stated that testosterone has been shown to inhibit cancer cell proliferation by counteracting the proliferative effects of endogenous hormones like DHEA.

Our results showed that high testosterone concentrations reduce the cell viability of LNCaP cell line after 48 hours of incubation.

As very high testosterone levels inhibit the proliferation of prostate cancer cells, this effect could be useful in the treatment of prostate cancer. However, these pathways have largely remained undiscovered at the cellular and molecular levels. thus, this study was performed to evaluate the effect of different testosterone concentrations on the viability of LNCaP prostate cancer cell line. High testosterone has an inhibitory effect on cell viability in the LNCaP cell line due to androgenic receptor (AR) downstream signaling or non-genomic AR activity. Furthermore, hormonal activation of the androgen receptor causes the receptor to self-stabilize, resulting in an increase in AR activity. As a result, in clinical practice, a therapeutic reduction in androgen levels is a clinical target that

would result in a drop in AR activity and, as a result, a reduction in AR-driven prostate cancer progression (Tiziana Siciliano *et al.*, 2022).

4.2 Effect of spironolactone on cell viability of LNCaP cell line:

The viability of the LNCaP cell line increased after treatment with a low concentrations of spironolactone, but decreased significantly when the concentration was increased to 1000 µg/ml. These results agree with Isla, (2016) who found that taking spironolactone was linked to a decreased risk of prostate cancer in a retrospective, matched cohort study (Isla et al., 2016). Also, this result agrees with Walsh, (1975) who found that spironolactone can suppress the level of plasma androgens. These findings could be attributed to the effect of spironolactone as an inhibitor of androgen synthesis, as found in a Walsh study in which spironolactone was given to castrated men with metastatic prostate cancer. This study found that plasma levels of testosterone, androstenedione, and dehydroepiandrosterone were significantly reduced, which could be related to spironolactone's ability to suppress adrenal androgen production (Walsh and Siiteri, 1975).

Also, the present study agree with Jody, 2020 who found that spironolactone binds non-selectively to progesterone and androgen receptors due to its anti-androgenic properties. The investigators found that men who had ever used spironolactone had significant 17% decreased odds of PCa. The effect was strongest among current users of the drug, who had significant 23% decreased odds of PCa. Spironolactone exposure

was significantly associated with 18% and 22% decreased odds of intermediate- and high-risk PCa, respectively. Spironolactone use had no effect on the risk of metastatic PCa diagnosis (Jody, 2020).

A direct dose-dependent decrease in prostate cancer was observed; meaning an increase in dose resulted in a decrease in the risk of prostate cancer. However, spironolactone exposure association with prostate cancer was not statistically significant in low-risk and advanced/ metastatic disease risk populations. Risk reduction of prostate cancer was also equal between symptomatic and asymptomatic patients. Spironolactone results in a 17% reduced risk of prostate cancer (Samhita, 2020).

4.3 Effect of docetaxel on cell viability of LNCaP cell line:

After a 24-hour incubation period, there are no significant ($P<0.05$) differences between the control group and concentrations of 31.25, 62.5, and 125 g/ml. The present study agree with Yang, 2019 who found that a low dose of docetaxel causes no apoptotic cell death, which is accompanied by senescence necrosis and mitotic catastrophe. Resistance to docetaxel is induced by dysregulation of AR signaling and transcriptional activity (Chongyi Yang, et al; 2019).

The decreased in the viability of the (LNCaP) cell line after treatment with all concentrations of docetaxel after 48 hours of incubation agrees with Yang et al., (2019) study that docetaxel suppresses the development of cultured prostate cancer cells in a dose-independent way. These findings are connected to the function of docetaxel as a microtubule inhibitor, which prevents microtubule disintegration by binding to -tubulin. (Yang *et al.*, 2019) As a result, by

arresting cells in the (G2/M phase) of the cell cycle, docetaxel causes cell death, which peaked at 24 and 48 hours. Docetaxel inhibited PI3K/Akt activation, lowered Bcl-2 levels, and enhanced caspase-3/9 activation, and it had a higher (inhibitory effect) on (AR-dependent LNCaP cells) than on AR-independent PC3 cells on growth suppression and apoptosis in prostate cancer cells. (Nehmé *et al.,* 2001)

4.4. Effect of spironolactone plus testosterone combination on the viability of LNCaP cell line:

Testosterone promotes the proliferation of LNCaP cells which drives prostate cancer growth via ligand-mediated activation of the androgen receptor (AR) (Morrissey *et al.,* 2020). Spironolactone possesses an antiandrogenic effect therefore decrease the promoting effect of testosterone on the growth of prostate cancer. These results agree with Patrick, (2017) who found that the treatment of castrated men with metastatic carcinoma of the prostate with spironolactone caused significant decrease in the plasma levels of testosterone, androstenedione and dehydroepiandrosterone due to the activity of spironolactone to suppresses adrenal androgen production and it has been suggested that it may be of benefit in the treatment of orchiectomized patients with advanced carcinoma of the prostate.

The reduction in the viability of the LNCaP cell line after 48 hours of exposure to testosterone –spironolactone combination reported by the current study agrees with Jeffrey and Marina (2015) study which found that despite historical evidence which suggested that testosterone is generally hazardous for men with active malignancies, testosterone therapy of the castrate-resistant patients may help in restoring hormone sensitivity and hence aid in transforming bad tumours into a

less aggressive phenotype. The development of prostate cancer cell is suppressed by high testosterone concentrations, whereas cell growth is stimulated by low testosterone doses.

4.5 Effect of different concentrations of spironolactone in combination with constant concentration (500 µg/ml) of docetaxel on the viability of LNCaP cell line:

In the current study the decrease in the viability of LNCaP cell line after 48 hours of exposure to different concentrations of spironolactone in combination to 500µg/ml of docetaxel agrees with Yang (2019) who study the effect of docetaxel on the regulation of proliferation and apoptosis of human prostate cancer cells and confirmed that docetaxel exerted an inhibitory effect on the growth of human prostate cancer cell line in a dose-independent manner and induced the apoptosis of these cells.

4.6 Effect of different concentrations of docetaxel in combination with constant concentration (500µg/ml) of spironolactone on the viability of LNCaP cell line:

In the present study the decrease in the viability of LNCaP cell line at high concentration and the increase in the viability of these cells at low concentrations of docetaxel after 24 hours of incubation as compared to 48 hours of incubation which result in a decrease in the viability of LNCaP cell line at all concentrations. These result agree with Dongbo *et al.* (2022) whose found that a preclinical study to repurpose spironolactone for enhancing chemotherapy response in bladder cancer moreover, spironolactone exhibited the potential

synergistic effects with other clinical chemotherapy regimens in bladder cancer cell lines.

Our results suggest that, the combination of spironolactone and docetaxel could be considered as a potent strategy in enhancing the efficacy of treatment, decreasing the docetaxel therapeutic dose and thereby lowering systemic toxicities and resistances.

4.7 Effect of testosterone plus spironolactone and docetaxel combination on the viability of LNCaP cell line after 48 hours of incubation:

In the present study the reduction in the viability of LNCaP cell line which was concentration dependent after 48 hours of exposure to the mixture containing 250 µg/ml and that containing 500 µg/ml of each agent (testosterone, spironolactone and docetaxel) agrees with Charles *et al.* (2020) study which demonstrated that androgen levels decline in some patients during treatment with docetaxel. Also, the results showed that a greater decline in androgens during docectaxel therapy is associated with a greater overall survival. The mechanisms of this phenomenon, include certain facets that the androgen receptor relies on microtubules for transport from the cytoplasm to the nucleus following ligand stimulation. Via microtubule stabilization, docetaxel impairs androgen transport after ligand activation, thus docetaxel's activity in CRPC can be attributed in part to its effect on AR transport. These observations suggest dual effects of androgen decline and microtubule targeting, when they occur, may convey additive, if not synergistic, benefits (Charles *et al.*, 2020)

Reference

- Alana Biggers, Medical News Today .What to know about cryotherapy for prostate cancer, 2021.
- Anju Verma, Megha Verma, and Anchal Singh , Animal tissue culture principles and applications , Animal Biotechnology. 2020 : 269–293. doi: 10.1016/B978-0-12-811710-1.00012-4.
- Arja Kaipainen *et al.* (2020) 'Testosterone accumulation in prostate cancer cells is enhanced by facilitated diffusion ', 79(13), pp. 1530–1542.
- Baker J, Ajani J, Florin Scoot, Dorte Winther, Migul Martin. *et al.* (2009) 'Docetaxel-related side effects and their management', European Journal of Oncology Nursing, 13(1), pp. 49–59.
- Bartosz Malinowski, Michał Wiciński, Nikola Musiała, Ilona Osowska, and Mateusz Szostak , Previous, Current, and Future Pharmacotherapy and Diagnosis of Prostate Cancer—A Comprehensive Review , 9(4): 161 , 2019.
- Bekelman JE, Rumble RB, Chen RC, Pisansky TM, Finelli A, Feifer A, et al. Clinically Localized Prostate Cancer: ASCO Clinical Practice Guideline Endorsement of an American Urological Association/American Society for Radiation Oncology/Society of Urologic Oncology Guideline. *J Clin Oncol*. 2018; 32: 3251-3258.
- Bosland, M. C. (2014) 'Testosterone treatment is a potent tumor promoter for the rat prostate', Endocrinology, 155(12), pp. 4629–4633.
- Bradley A. Maron, M.D. and Jane A. Leopold, M.D.National Library of Medicine, Aldosterone Receptor Antagonists: Effective but Often Forgotten doi: 10.1161/CIRCULATIONAHA.109.895235 , 121(7): 934–939.2011.
- Chad R Tracy, MD; Chief Editor: Edward David Kim, MD, FACS, Prostate

Cancer, Medscape 2021.

- Charles J Ryan, Sandipan Dutta, William K Kelly, Carly Russell, Eric J. Small, Michael J. Morris, Mary-Ellen Taplin, Susan Halabi, and Alliance for Clinical Trials in Oncology Genitourinary Committee. Androgen Decline and Survival During Docetaxel Therapy in metastatic Castration Resistant Prostate Cancer (mCRPC). 2020 Mar; 23(1): 66–73.
- Chaudhary, M. V., & Singh, P., (2019). Cell Line : a Review. May, 254–263.
- Chongyi Yang , Weijie Zhang , Jie Wang, Pengpeng Chen and Jiangjiang Jin, National Library of Medicine, Effect of docetaxel on the regulation of proliferation and apoptosis of human prostate cancer cells, 19(5):3864-3870. doi: 10.3892/mmr.2019.9998. Epub 2019.
- Claire Gillespie, sciencing. How Does Sonication Work? 2018.
- Corinne O'Keefe Osborn, medically reviewed by Alan Carter, Pharm.D. Healthline Your Guide to Anti-Androgens, 2018.
- Corvol P, Michaud A, Menard J, Freifeld M, and Mahoudeau J. Endocrinology. 1975 Jul;97(1):52-8. doi: 10.1210/endo-97-1-52.
- Cosimo De Nunzio, Gerald L. Andriole, Ian M. Thompson Jr & Stephen J. Freedland, EURPEAN UROLOGY FOCUS, Smoking and Prostate Cancer: A Systematic Review , 1 (1) , P28-38, 2015 .
- Dalvinder Mandair, Roberta Elisa Rossi, Marinos Pericleous, Tara Whyand & Martyn Evan Caplin, Prostate cancer and the influence of dietary factors and supplements: a systematic review (2014) .
- Daniyal, Zamir Ali, Muhammad Akram, HM Asif , Sabria sultana. et al. (2014) ‘MINI-REVIEW Epidemiology , Etiology , Diagnosis and Treatment of Prostate Cancer’, 15, pp. 9575–9578.

- Dongbo Xu; Qiang Cao; Li Wang; Jianmin Wang; Bo Xu; Kristopher Attwood; Lei Wei ; Gary J. Smith; Eriko Katsuta ; Kazuaki Takabe ; Gurkamal Chatta; Khurshid A. Guru; David W. Goodrich and Qiang J. Li, A Preclinical Study to Repurpose Spironolactone for Enhancing Chemotherapy Response in Bladder Cancer (2022) 21 (5): 786–798.
- Dwight E. Lynn, in Encyclopedia of Insects (Second Edition), cell culture,2009.
- Fang D, Zhou L. Androgen deprivation therapy in nonmetastatic prostate cancer patients: Indications, treatment effects, and new predictive biomarkers. Asia Pac J Clin Oncol 2019;15(3):108-20.
- George R. Thompson, Prasanth N. Surampudi, and Alex Odermatt. Gynecomastia and hypertension in a patient treated with Posaconazole 2020 Dec; 8(12): 3158–3161.
- Ginah Nightingale, PharmD, BCOP and Jae Ryu, PharmD, BCOP, BCPS , US National Library of Medicine National Institutes of Health , Cabazitaxel (Jevtana) A Novel Agent for Metastatic Castration-Resistant Prostate Cancer 37(8), 2012.
- Guoqiang Zhang , Xinrui Zhao , Xueliang Li 2 , Xiulan Sun 3 , Jingwen Zhou 1 2 , Guocheng Du 2 , Jian Chen Zhang, G. et al. (2019) 'Application of cell culture techniques in cultured meat-a review', Sheng wu gong cheng xue bao = Chinese journal of biotechnology, 35, pp. 1374–1381. doi: 10.13345/j.cjb.190138.
- Herbert Lepor, MD and Neal D Shore, MD, FACs, review in Urology, LHRH Agonists for the Treatment of Prostate Cancer: 2012, 14(1-2): 1–12, 2012.

- Isla S. Mackenzie,Steven V. Morant,Li Wei,Alastair M. Thompson and Thomas M. MacDonald (2016) . British Pharmacological society Journal. Spironolactone use and risk of incident cancers: a retrospective, matched cohort study, Citations: 27.
- Jack H. Mydlo, Ciril J. Godec Prostate Cancer: Science and Clinical Practice Second Edition p. 534, 2015.
- Jacques-Pierre Moreau, Patrick Delavault, Joëlle Blumberg, National Library of Medicine Luteinizing hormone-releasing hormone agonists in the treatment of prostate cancer: a review of their discovery, development, and place in therapy, 28(10):1485-508, 2006.
- Jody A. Charnow Spironolactone Use Associated With Reduced Prostate Cancer Risk, 2020.
- Jyoti D. Patel, MD, FASCO, Neeraj Agarwal, MD, Eren Berber, MD, Prostate Cancer: Risk Factors and Prevention, Approved by the Cancer.Net Editorial Board, 09/2020.
- Katherine A. Lyseng-Williamson and Caroline Fenton , Docetaxel: a review of its use in metastatic breast cancer 2005, 65 (17) .
- Kerri Beckmann, Hans Garmo, Bertil Lindahl, Lars Holmberg, Pär Stattin, Jan Adolfsson, J. Kennedy Cruickshank & Mieke Van Hemelrijck , Spironolactone use is associated with lower prostate cancer risk: a population-wide case-control study Prostate Cancer and Prostatic Diseases volume 23, pages527–533 (2020)
- Laurel Kelly, Consumer Health: Are you at risk for prostate cancer? Mayo Clinic; 2021.

- Le Wang, Bin Lu, Mengjie He, Youqing Wang , Zongping Wang and Lingbin Du Prostate Cancer Incidence and Mortality: Global Status and Temporal Trends in 89 Countries From 2000 to 2019, 2022.
- Leon P. Bignold, in Principles of Tumors (Second Edition), Chapter 15 - Specific aspects of cytotoxic and hormonal drug therapies , 2020.
- Lewis, D. D. and Cropp, C. D. (2020) 'The impact of African ancestry on prostate cancer disparities in the era of precision medicine', Genes, 11(12), pp. 1–28.
- Lisa A., Prostate Cancer: Thriving Through Treatment to Recovery, p 8, 2019.
- Litwin, M. S. and Tan, H. J. (2017) 'The diagnosis and treatment of prostate cancer: A review', JAMA - Journal of the American Medical Association, 317(24), pp. 2532–2542.
- Lu, Xinxing , Yang, Feiya ,Chen, Dexi ,Zhao, Qinxin ,Chen, Dong ,Ping, Hao Xing, Nianzeng , Quercetin reverses docetaxel resistance in prostate cancer via androgen receptor and PI3K/AKT signaling pathways , International Journal of Biological Sciences, 2020, 16(7) .
- Luhur A, Klueg KM, Roberts J and Zelhof AC. Thawing, Culturing, and Cryopreserving Drosophila Cell Lines. JoVE 2019;(146):e59459.
- Matt Carter, J. C. S., (2015). Cell culture techniques. Principles and Techniques of Biochemistry and Molecular Biology, Sixth Edition, 71–102.
- Maurie Markman, Cancer treatment center of America. Prostate cancer stages, 2021.
- Meerloo, J. van, Kaspers, G. J. L., & Cloos, (2011). Cancer Cell Culture, MTT assay. Methods Mol Biol, 731(1), 79–91
- Michael F Leitzmann, Sabine Rohrmann, Risk factors for the onset of

prostatic cancer: age, location, and behavioral correlates , 1 (11) , 2012.

- Michel Bolla and Hendrik van Poppel, management of prostate cancer 2017. pp.1
- Miyamoto H., Messing, E. M. and Chang, C. (2004) 'Androgen deprivation therapy for prostate cancer: Current status and future prospects', Prostate, 61(4), pp. 332–353.
- Mohammad Imran, Sadaf Saleem, Aiswarya Chaudhuri, Javed Ali and Sanjula Baboota. Journal of Drug Delivery Science and Technology. Docetaxel: An update on its molecular mechanisms, therapeutic trajectory and nanotechnology in the treatment of breast, lung and prostate cancer Volume 60, December 2020, 101959
- Morgentaler, A. and Traish, A. M. (2009) 'Shifting the Paradigm of Testosterone and Prostate Cancer: The Saturation Model and the Limits of Androgen-Dependent Growth', European Urology, 55(2), pp. 310–321.
- National cancer institute, Genetics of Prostate Cancer (PDQ®)–Health Professional Version, 2022.
- Nicole G. Farha and Anup Kasi. Docetaxel, 2021.
- Omesh Singh and Srinivasa Rao Bolla. National Library of medicine. Anatomy, Abdomen and Pelvis, Prostate, 2021.
- Omesh Singh; Srinivasa Rao Bolla. National library of medicine. Anatomy, Abdomen and Pelvis, Prostate, 2021.
- Onur Uysal , Tugba Sevimli , Murat Sevimli and Sibel Gunes , ResearchGate, Cell and Tissue Culture. Omics Technologies and Bio-Engineering (pp.391-429) DOI:10.1016/B978-0-12-804659-3.00017-8, January 2018.
- P C Walsh and P K Siiteri, National Library of medicine , Suppression of

plasma androgens by spironolactone in castrated men with carcinoma of the prostate, 114(2):254-6. doi: 10.1016/s0022-5347(17)67001-0, 1975.

- Patrick C.Walsh and Pentti K.Siiteri, The Journal of Urology, Suppression of Plasma Androgens by Spironolactone in Castrated Men with Carcinoma of the Prostate, Volume 114, Issue 2, Pages 254-256, 2017.
- Perdana , N. R. AgusRizal ,Chaidir ,A Rainy Umbas . (2016) ‘ the risk factor of prostate cancer and its prevention’ , Acta medica Indonesiana, 48(3), pp. 228–238.
- Philip Thornton, DipPharm.Drugs.com Bicalutamide 2021.
- Philippeos, C., Hughes, R. D., Anil Dhawan, A., & Mitry, R. R., (2012). Human Cell Culture Protocols. 806, 301–336.
- Pienta , K. J. (2001) ‘Preclinical mechanisms of action of docetaxel and docetaxel combinations in prostate cancer’, Seminars in Oncology, 28(4 SUPPL. 15), pp. 3–7.
- Rafael A. Kaliks and Auro Del Giglio (2008) ‘management of advanced prostate cancer.
- Rajanna A. Novel approach to target cancer stem cells for therapy. Med Hypotheses. 2016;88:83–5.
- Rawla, Prashanth (2019) Epidemiology of Prostate Cancer, World J Oncol.;10(2):63-89.
- Reddy, Androgen Biosynthesis Inhibitor, 2021.
- Richard R. Barakat, Maurie Markman, Marcus Randall, Principles and Practice of Gynecologic Oncology, administration and dosage, 2009 pp. 422.
- Rick Alteri and Mamta Kalidas, American cancer society. Hormone Therapy for Prostate Cancer, 2021.
- Samhita Vitta Spironolactone - A Hypertension Diuretic Drug Reduces

Prostate Cancer Risk, 2020.

- Santhanam Sundar and Peter D Dickinson , PMC PubMed Central® Spironolactone, a possible selective androgen receptor modulator, should be used with caution in patients with metastatic carcinoma of the prostate , 2012, doi: 10.1136/bcr.11.2011.5238.
- Sarah A. Holstein and Raymond J. Hohl. Pharmacology and Therapeutics. Prostate cancer ,2009.
- Sebastian Student, Tomasz Hejmo, Aleksandra Poterała-Hejmo, Aleksandra Leśniak and Rafał Bułdakc. (2020) Anti-androgen hormonal therapy for cancer and other diseases, European Journal of Pharmacology, Volume 866.
- Stephan Madersbacher, Antonio Alcaraz, Mark Emberton, Peter Hammerer, Anton Ponholzer, Fritz H. Schröder and Andrea Tubaro. The influence of family history on prostate cancer risk: implications for clinical management, 2010.
- Stephen W. Leslie; Taylor L. Soon-Sutton; Hussain Sajjad and Larry E. Siref. Prostate Cancer, 2022.
- Stephen W. Leslie; Taylor L. Soon-Sutton; Hussain Sajjad; Larry E. Siref, Prostate Cancer, StatPearls, 2021.
- Strober, W., (2015). Trypan Blue Exclusion Test of Cell Viability. Current Protocols in Immunology, 111(1), A3.B.1-A3. B.3.
- Suzuki , Y. and Tokuda , Y. (2001) 'Docetaxel', Gan to kagaku ryoho. Cancer & chemotherapy, 28(10), pp. 1363–1367. d
- Tan Guan Lim Lincoln, Prostate cancer may be the third most prevalent cancer amongst men in Singapore, but early detection and treatment can help save lives, 2019.

- Tannock, I. F. (2001) 'Management of metastatic prostate cancer: recent advances, clinical perspectives and future prospects', Journal of B.U.ON., 6(3), pp. 227–230.
- Tiziana Siciliano, Ulrich Sommer, Alicia-Marie K. Beier, Matthias B. Stope, Angelika Borkowetz , Christian Thomas and Holger H. H. Erb, Current Issues in Molecular Biology , The Androgen Hormone-Induced Increase in Androgen Receptor Protein Expression Is Caused by the Autoinduction of the Androgen Receptor Translational Activity, 2022, 44, 597–608. https://doi.org/10.3390/ cimb44020041.
- Vasilia angnostopoulou , Losif Pediaditakis , Saad AL Kahtani , Saud A.Alarafi, Eva mari. *et al.* (2013) 'Differential effects of dehydroepiandrosterone and testosterone in prostate and colon cancer cell apoptosis: The role of nerve growth factor (NGF) receptors', Endocrinology, 154(7), pp. 2446–2456.
- Waltham.mass (2014) Treating advanced prostate cancer., Health news (Waltham, Mass.),14(4), pp. 7.
- Wendy Bijoux, Emilie Cordina-Duverger, Soumaya Balbolia, Pierre-Jean Lamy, Xavier Rebillard, Brigitte Tretarre, Sylvie Cenee and Florence Menegaux. Occupation and prostate Cancer risk: results from the epidemiological study of prostate cancer (EPICAP) Journal of Occupational Medicine and Toxicology. (2022) **17**, Article number: 5.
- Youyuan Xu, Shao-Yong Chen, Kenneth N Ross and Steven P Balk, National Library of Medicine pubmed , Androgens induce prostate cancer cell proliferation through mammalian target of rapamycin activation and post-transcriptional increases in cyclin D proteins, 2006, 1;66(15):7783-92. doi: 10.1158/0008-5472.CAN-05-4472.

Appendices

Table 3.1 Effect of testosterone on the viability of LNCaP cell line after 24 hours of incubation

Comparison of control group with testosterone concentrations µg/ml on LNCaP cell line after 24 hours of incubation	Mean Difference (I-J)	Std. Error	p. value
control vs. 31.25	0.028000	0.028369	0.337
control vs. 62.5	-0.009000	0.028369	0.755
control vs. 125	-0.013000	0.028369	0.653
control vs. 250	-0.061333	0.028369	0.045*
control vs. 500	-0.019667	0.028369	0.498
control vs. 1000	-0.039667	0.028369	0.180

Table 3.2 Effect of testosterone on the viability of LNCaP cell line after 48 hours of incubation:

Comparison of control group with testosterone concentrations µg/ml on LNCaP cell line after 48 hours of incubation	Mean Difference (I-J)	Std. Error	p.value
control vs. 31.25	0.037667	0.030414	0.232
control vs. 62.5	0.004333	0.030414	0.888
control vs. 125	-0.042667	0.030414	0.179
control vs. 250	0.073000	0.030414	0.028*

control vs. 500	0.178667	0.030414	0.000*
control vs. 1000	0.182667	0.030414	0.000*

Table 3.3 Effect of spironolactone on the viability percentage of LNCaP cell line after 24 of incubation

Comparison of control group with spironolactone concentrations µg/ml on LNCaP cell line after 24 hours of incubation	Mean Difference (I-J)	Std. Error	p.value
control vs. 31.25	-0.030333	0.035067	0.399
control vs. 62.5	-0.014667	0.035067	0.681
control vs. 125	-0.020000	0.035067	0.576
control vs. 250	0.021333	0.035067	0.551
control vs. 500	0.052000	0.035067	0.156
control vs. 1000	0.062667	0.035067	0.092

Table 3.4 Effect of spironolactone on the viability percentage of LNCaP cell line after 48 hours of incubation

Comparison of control group with spironolactone concentrations µg/ml on LNCaP cell line after 48 hours of incubation	Mean Difference (I-J)	Std. Error	p.value
control vs. 31.25	0.012333	0.032752	0.711
control vs. 62.5	-0.062333	0.032752	0.074
control vs. 125	0.024667	0.032752	0.462
control vs. 250	-0.006667	0.032752	0.841
control vs. 500	0.002667	0.032752	0.936
control vs. 1000	0.039333	0.032752	0.246

Table 3.5 Effect of docetaxel on the viability Percentage of LNCaP cell line after 24 hours of incubation

Comparison of control group with docetaxel concentrations µg/ml on LNCaP cell line after 24 hours of incubation	Mean Difference (I-J)	Std. Error	P.value
control vs. 31.25	0.00000	0.02887	1.000
control vs. 62.5	-0.00667	0.02887	0.820
control vs. 125	-0.00133	0.02887	0.964
control vs. 250	0.08233	0.02887	0.011*
control vs. 500	0.12667	0.02887	0.000*
control vs. 1000	0.14800	0.02887	0.000*

Table 3.6 Effect of docetaxel on the viability Percentage of LNCaP cell line after 48 hours of incubation

Comparison of control group with docetaxel concentrations µg/ml on LNCaP cell line after 48 hours of incubation	Mean Difference (I-J)	Std. Error	p.value
control vs. 31.25	0.299000	0.024438	0.000*
control vs. 62.5	0.296667	0.024438	0.000*
control vs. 125	0.303333	0.024438	0.000*
control vs. 250	0.305333	0.024438	0.000*
control vs. 500	0.304333	0.024438	0.000*
control vs. 1000	0.303667	0.024438	0.000*

Table 3.7 Effect of spironolactone plus testosterone combination on the viability percentage of LNCaP cell line after 24 hours of incubation

Comparison of control group with testosterone plus spironolactone combination concentrations µg/ml on LNCaP cell line after 24 hours of incubation	Mean Difference (I-J)	Std. Error	p.value
control vs. 31.25	0.162667	0.015191	0.000*
control vs. 62.5	0.051000	0.015191	0.004*
control vs. 125	-0.012000	0.015191	0.440
control vs. 250	-0.044333	0.015191	0.010*
control vs. 500	0.158333	0.015191	0.000*
control vs. 1000	0.219333	0.015191	0.000*

Table 3.8 Effect of spironolactone plus testosterone combination on the viability percentage of LNCaP cell line after 48 hours of incubation

Comparison of control group with testosterone plus spironolactone combination concentrations µg/ml on LNCaP cell line after 48 hours of incubation	Mean Difference (I-J)	Std. Error	p.value
control vs. 31.25	0.090333	0.042093	0.047*
control vs. 62.5	0.069000	0.042093	0.120
control vs. 125	0.072000	0.042093	0.105
control vs. 250	0.065000	0.042093	0.141
control vs. 500	0.079000	0.042093	0.078
control vs. 1000	0.072000	0.042093	0.105

Table 3.9 Effect of spironolactone different concentrations combined to 500 µg/ml docetaxel on the viability of LNCaP cell line after 24 hours of incubation

Comparison of control group with docetaxel 500 µg/ml plus different concentrations of spironolactone combination on LNCaP cell line after 24 hours of incubation	Mean Difference (I-J)	Std. Error	p.value
control vs. 500+15.625	-0.009500	0.047396	0.844
control vs. 500+31.25	-0.023167	0.047396	0.631
control vs. 500+62.5	-0.001167	0.047396	0.981
control vs. 500+125	-0.106500	0.047396	0.038*
control vs.500+250	-0.132167	0.047396	0.013*
control vs. 500+500	-0.124833	0.047396	0.017*

Table 3.10 Effect of spironolactone different concentrations combined to 500 µg/ml docetaxel on the viability of LNCaP cell line after 48 hours of incubation

Comparison of control group with docetaxel 500 µg/ml plus different concentrations of spironolactone combination on LNCaP cell line after 48 hours of incubation	Mean Difference (I-J)	Std. Error	p.value
control vs. 500+15.625	0.196833	0.007798	0.000*
control vs. 500+31.25	0.196167	0.007798	0.000*
control vs. 500+62.5	0.196833	0.007798	0.000*
control vs. 500+125	0.183167	0.007798	0.000*
control vs.500+250	0.184167	0.007798	0.000*
control vs. 500+500	0.197500	0.007798	0.000*

Table 3.11 Effect of docetaxel different concentrations combined to 500µg/ml spironolactone on the viability of LNCaP cell line after 24 hours of incubation

Comparison of control group with spironolactone 500 µg/ml plus different concentrations of docetaxel combination on LNCaP cell line after 24 hours of incubation	Mean Difference (I-J)	Std. Error	p.value
control vs. 500+15.625	-0.084167	0.0466	0.088
control vs. 500+31.25	0.069500	0.0466	0.154
control vs. 500+62.5	0.145167	0.0466	0.006 *
control vs. 500+125	0.146167	0.0466	0.006 *
control vs.500+250	0.175833	0.0466	0.001*
control vs. 500+500	0. 0.2151	0.0466	0.000*

Table 3.12 Effect of docetaxel different concentrations combined to 500µg/ml spironolactone on the viability of LNCaP cell line after 48 hours of incubation

Comparison of control group with spironolactone 500 µg/ml plus different concentrations of docetaxel combination on LNCaP cell line after 48 hours	Mean Difference (I-J)	Std. Error	p.value
control vs. 500+15.625	0.135833	0.020901	0.000*
control vs. 500+31.25	0.179167	0.020901	0.000*
control vs. 500+62.5	0.183167	0.020901	0.000*
control vs. 500+125	0.190833	0.020901	0.000*
control vs.500+250	0.193833	0.020901	0.000*
control vs. 500+500	0.196167	0.020901	0.000*

Table 3.13 Effect of spironolactone plus testosterone and docetaxel combination on the viability percentage of LNCaP cell line after 48 hours of incubation

Comparison of control group with spironolactone plus testosterone and docetaxel combination on LNCaP cell line after 48 hours	Mean Difference (I-J)	Std. Error	p.value
control vs. 15.625+15.625+15.625	0.050333	0.046731	0.296
control vs. 31.25+31.25+31.25	0.085667	0.046731	0.084
control vs. 62.5+62.5+62.5	0.146000	0.046731	0.006*
control vs. 125+125+125	0.162667	0.046731	0.003*
control vs. 250 +250+250	0.214000	0.046731	0.000*
control vs. 500+500+500	0.387000	0.046731	0.000*