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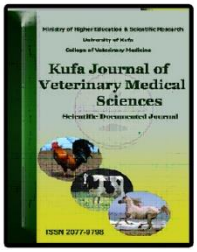

Effects of alcoholic extract of *panax ginseng* Seeds and Tamoxifen citrate drug on some fertility parameters and histopathological changes in testes of mice treated with doxorubicin

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Abstract

The objective of this revision to examine the properties of *panax gensing* extract and tamoxifen citrate drug on fertility of male mice testes treated with Doxorubicin, the plant was extracted by using 70%ethanolic alcohol. The mice were divided into five groups: The (T1) was given 300 mg / kg of extract, (T2) was given 150mg/kg of extract and 0.2mg/kg of Tamoxifen, (T3) was given Tamoxifen 0.4 mg/kg, (T4) was given distilled water. Starting from one week, T1,T2 and T3 were intraperitoneally administered 0.15mg/kg of doxorubicin twice weekly for 5 week. (T5) was treated 0.15mg/kg of doxorubicin twice weekly. Results appeared the group given extract display important increase in motility index and turdidimetric analysis of sperms, and decrease in percentage of dead sperm and abnormalitites, the group treated with extract and tamoxifen citrate drug showed important decrease in percentage of dead sperm and abnormalities and important increase in turdidimetric analysis of sperms as compared with tamoxifen group. The histopathological section of testis of doxorubicin group noticed severe degenerative changes in the wall of seminiferous tubules also atrophy in the spermatogonia and Sertoli cells, the section of extract group exhibited increase in number of spermatid and spermatozoa, the section of tamoxifen appeared edema in the interstitial tissue and congestion of blood vessels, the mix of panax gensing extract and tamoxifen citrate displayed decrease in redness of vessels and tissue. From this revision established the extract of *panax gensing* seeds play important role in increase fertility of male mice by enhance spermatogenesis and have the ability to decrease side effect of tamoxifen citrate.

Key words: *panax ginseng*, Tamoxifen citrate, doxorubicin and fertility.

تأثير المستخلص الكحولي لبذور نبات الجنسنغ باناكس ودواء تاموكسيفين سيترات على بعض قياسات الخصوبة والتغيرات التشريحية المرضية في في خصى الفئران التي عولجت مع دوكسوروبيسين

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الخلاصة:

الهدف من هذه الدراسة لمعرفة تأثير مستخلص الباناكس جينسنغ ودواء سترات تاموكسيفين على خصوبة الخصى في ذكور الفئران المعاملة ب دوكسوروبيسين، تم استخلاص النبات باستخدام 70٪ كحول الايثانول. قسمت الفئران إلى خمس مجموعات : أعطيت المجموعة الاولى300 ملغ / كغ, من المستخلص النباتي، أعطيت المجموعة الثانية150 ملغم / كغم من المستخلص و0.2ملغم/كغم من تاموكسيفين، أعطيت المجموعة الثالثة تاموكسيفين 0.4 مغ / كغ، بينما المجموعة الرابعة جرعت ب الماء المقطر. بدءا من أسبوع الاول المجاميع، الاولى والثانية الثالثة اعطيت دوكسوروبسين 0.15ملغم/ كغم تحت الخلب مرتين أسبوعيا لمدة خمسة اسابيع. اعطيت المجموعة الخامسة دوكسوروبيسين 0.15ملغم/ كغم مرتين أسبوعيا. أظهرت النتائج زيادة مهمة في مؤشر القدرة على الحركة وتحليل الكثافة وكدورة القياسية للحيوانات المنوية، وانخفاض في نسبة الحيوانات المنوية الميتة والتشوهات، أظهرت المجموعة التي تلقت العلاج مع المستخلص ودواء سترات تاموكسيفين انخفاضا هاما في نسبة الحيوانات المنوية وتشوهات والزيادة الهامة في تحليل الكثافة وكدورة القياسية للحيوانات المنوية بالمقارنة مع مجموعة تاموكسيفين. اضهرت التغيرات النسيجية للخصية لل مجموعة المعالجة ب دوكسوروبيسين انتكاسات شديدة في جدار الأنابيب المنوية مع ضمور أيضا في خلايا المنوية وخلايا سيرتولي، اضهرت المجموعة المعالجة بالمستخلص زيادة في عدد النطف والحيوانات المنوية، بينما اضهرت التغييرات النسيجية ل عقار تاموكسيفين احتقان في الأوعية الدموية، بينت المجموعة التي اعطيت مستخلص باناكس جينسغ وسترات تاموكسيفين قلة في احتقان الأوعية الدموية في النسيج الخلالي. نستنتج من هذه الدراسة ان المستخلص لبذور باناكس جينسغ تلعب دورا هاما في زيادة خصوبة ذكور الفئران عن طريق تعزيز تكوين الحيوانات المنوية ولها القدرة على تقليل الآثار الجانبية للتاموكسيفين سيترات.

Introduction:

Medical herbal plant, its principles for healthiness precaution needs (1) in addition its excessive needed in the rising countries for major in healthcare since of their extensive medical activities, greater care restrictions and smaller prices (2) *Panax* plant fits to Araliaceaes family's, plant of *panax*, used curatively for several of ages in China, (3) it is recognized as an adaptogene and a healing tonic that is extensively used in Chinese medication (4) Heterogeneous usages for *Panax* plant include sterility, liver diseases, menopausal, and in erection defect.(5). The plant has long been traditionally had functions by supporting immunity, giving nourishment and improving health from tiredness (6). The effect of ginseng has been established recently by numerous detectives, Ginseng plant hold several pharmacological constituents ginsenosides (saponins), poly-acetylenes, poly-phenolic composites and polysaccharide, the most important contains of plant are ginsenosides which is nearly major pharmaceutical action of plants returned to ginsenosides (7).

Doxorubicin (adriamycin) an anti tumour antibiotic is effective against solid and non- solid malignant tumour. It is known to produce reproductive toxicity, Doxorubicin exhibits profound toxicity to the reproductive system, adversely affecting male fertility (8). Doxorubicin causes acut progressive alterations in tissues, decrease in the mass of seminiferous cells and wideness. (9),

Tamoxifen citrate is considered non-steroidal anti-estrogenic complex which act as competitive antagonist for estrogen receptors and have a diverse antagonist with agonist influence, estrogen blockers action incompletely obstruct the effect of its agonist action, they also give certain estrogenic properties , are approved to the idiopathic oligo-azoospermia in animals, it, react with negative feed-back hypothalamus and pituitary hormones, lead to increase gonadotropin hormones (GnRH) and luteal hormone discharge, which is encourages Leydig tissues of testis;

lead to enhance testosterone hormone creation, thus improving spermatogenesis, an enhancement in fertility (10), The target of this effort is to revision the effects of panax gensing extract and tamoxifen citrate drug on some parameters of sperms and some histopathological changes on mice testes treated with Doxorubicin.

Materials and Methods

Extraction of *Panax ginseng* seeds:

The shade dried plant was grinded by an electric grinder into powder. Sixty grams from the grinded crude powder was mixed with 100 ml of 70%ethanolic alcohol (600 mg/ml) and put in flask, The flask was placed on hot plate magnetic stirrer . The temperature of extraction was 40-45c°. The solution was left stirring for 72 hours and then sieved by using sterialguaze to get rid of coarse particles. The solution then filtered through Whitmannfliter . The filtrate was poured in clean and sterile petridishes (12x2 cm) and kept in incubator at temperature of 45c° until dryness (11**).**

Animals and Treatment: fifty thirty adult albino male mice (30-33) gm were purchased from pharmacy collage/ university of Baghdad and maintained in the animal house. Mice were housed plastic cage 30x10x10 cm placing in the house till the start of experiment. Regular food and water was spontaneously obtainable. The mice were divided into five groups (10 mice in each one) the period of treatment was five week: The first (T1) was treated with a daily300 mg / kg body weight of *Panax ginseng* given orally, The second group (T2) was treated with a daily by150 mg/kg of *Panax ginseng* extract and 0.2mg/kg of Tamoxifen for five week, The third group (T3) was treated with a daily by Tamoxifen 0.4 mg/kg given orally, The fourth group (T4) was treated with a daily by distilled water (negative control group). Starting from one week, T1,T2 and T3 were intraperitoneally administered 0.15mg/kg of doxorubicin twice weekly for 5 week. The fifth group (T5) was treated only by intraperitoneally 0.15mg/kg of doxorubicin twice weekly for 5 week (positive control group).

Parameters used in this experiments:

Testosterone measurement:

Blood testers were examined for testosterone by consuming the enzymes-linke immune-sorbent test (ELISA) by consuming the Fortress kits.

Collection of the semen:

The caudal epididymis's were removed from the testes and put on the mesh paper then cut to accumulate the semen.

Semen Examination

Motility, Lag times, Velocities and moving fraction of sperms: these were measurement directly after the semen accumulation. the method of Spectrophotometer was used to estimate Lag times, Velocities, move of fraction of sperm according to (12). Sperms were pressed from epididymis and put on warmed slide then 2 droplets of 2.9 % sodium citrates was additional, then put the cover on slide and examined under the microscope. 10 areas in the slide were arbitrarily examined. 100 sperms were measured to access the motility. Percentages of motile were determined by divided the numbers of motile sperm on the whole numbers of calculated sperm (i.e. 100) (13).

Measurement dead and life sperms: this was determinate by putting 2 droplets of warms eosin to nigrosins stains to sperms on warm slide, then the slur was complete and dry in air;

then the slides were studied under the microscope. Live cells of sperms were don't take the stain while the stain was taken in dead cells. (14).

Measurement of morphology: this was determinate by putting 2 droplets of warms eosin to nigrosins stains to the sperms on warm slide, then slur complete and dry in air; then the slides were studied under the microscope under the microscope. 5 fields under the microscope were detected and anomalous sperm was calculated by divided it on the total numbers of sperm x100.

Histopathological Investigation: Testes of mice were reserved and put in 10% formalin, the samples then washed and put in alcohol marks. These samples were clean with xylene, put in paraffins, divided at four to six micron width and marked with Hematoxylen / Eosin (H &E) (15).

Statistical analysis: the programs SAS (16) was taken to analysis this study the effect of different preserved in adjective studies and the significant between medium was compared with less significant LSD.

Results

Motility index, Dead sperm% and Abnormality %:

This study showed the group treated with extract of panax ginseng Seeds display important P<0.05 rise in motility index, and reduction in percentage of dead sperm and abnormalitites as compared with control and other groups, whereas the group treated with extract of panax ginseng Seeds and tamoxifen citrate drug showed significant decrease P<0.05 in calculation of dead sperms with its abnormalities as compared with group treated with tamoxifen drug as in table (1).

Table (1): Effect of of alcoholic extract of panax ginseng Seeds , Tamoxifen citrate drug, doxorubicin and distilled water on percentage of dead sperms, percentage sperms abnormalities and motility index of mice.

Groups	Motility index	Dead sperm%	Abnormality %
T1	2.99 ± 0.070 A	19.09±3.01 A	12.33±4.59 A
T2	2.68 ± 0.080 B	20.10 ± 4.91 A	15.10 ± 4.66 B
T3	2.01 ± 0.051 C	23.16 ± 7.20 B	20.15 ± 4.10 C
T4	1.18 ± 0.051 D	20.14±5.00 C	15.09±4.05 B
T5	0.98 ± 0.035 E	35.46±4.07 D	38.11±4.20 D

*Data taken as mean ± SE *Altered capital letter mean important alteration (P□0.05) among column number.

Velocity, Fraction of rapidly moving sperm and Lag time second of sperms and testosterone:

Result of table (2) referred to important P<0.05 surge in turbidi-metric examination of sperms (Velocity, Fraction of rapidly moving sperm and Lag time second of sperms) in group treated with alcoholic extract of panax ginseng Seeds as compared with control and other groups, furthermore the turbidimetric analysis of sperm in group treated with extract of panax

ginseng Seeds and tamoxifen citrate drug showed significant increase P<0.05 as compared with group treated with tamoxifen drug. Testosterone hormone in groups (T1,T2 and T3) showed significant increase P<0.05 as compared with T4 and T5

Table (2): The Velocity µm/sec, Fraction of rapidly moving sperm and Lag time second of sperms in male mice under loading doses of extract of panax ginseng Seeds , Tamoxifen citrate drug and doxorubicin for 35 days.

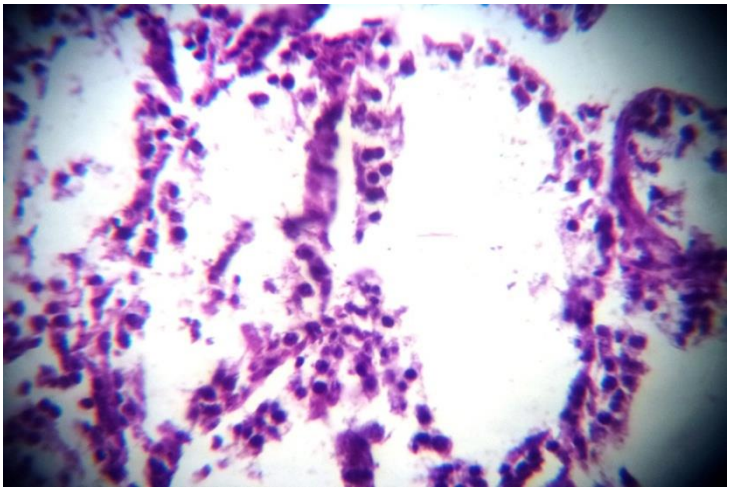
Groups / Parameters	T1	T2	T3	T4	T5
Velocity µm/sec	14.05 ± 1.06 A	12.04 ± 0.96 B	11.31 ± 0.62 C	9.10 ± 0.099 D	7.01 ± 0.042 E
Fraction of rapidly moving sperm	0.184 ± 0.009 A	0.169 ± 0.011 B	0.150 ± 0.008 C	0.0167 ± 0.005 D	0.0125 ± 0.003 E
Lag time second	0.69 ± 0.005 A	0.71 ± 0.002 B	0.75 ± 0.004 C	1.94 ± 0.0083 D	2.01 ± 0.099 E
Testosterone	8.7 ± 0.075 A	8.0 ± 0.071 A	7.9 ± 0.069 A	6.1 ± 0.08 B	1.2 ± 0.032 C

*Data taken as mean ± SE

*Altered capital letter referred to important alteration (P□0.05) among raw number.

Histopathological changes:

The histopathological section of testis in mouse treated with intraperitoneally 0.15 mg/kg of doxorubicin twice daily for five weeks noticed severe degenerative changes in the wall of seminiferous tubules also atrophy in the spermatogonia and Sertoli cells, whereas the section treated with *panax gensing* alcoholic extract exhibited better results by increase in number of spermatid and spermatozoa and mild changes when compared with other treated groups, but the section in mouse treated with tamoxifen citrate drug appeared edema in the interstitial tissue and congestion of blood vessels, in the other hand the section in testis of mouse treated with panax gensing extract and tamoxifen citrate drug showed improved by presence very few number of spermatozoa when compared with negative control group, in addition to decrease congestion of blood vessels in the interstitial tissue.



Figure(1): Histopathological section in testis of mouse treated with intraperitoneally 0.15 mg/kg of doxorubicin twice daily for five weeks showed Severe degenerative changes in the wall of seminiferous tubules also atrophy in the spermatogonia and Sertoli cells (H&E. X 100). .

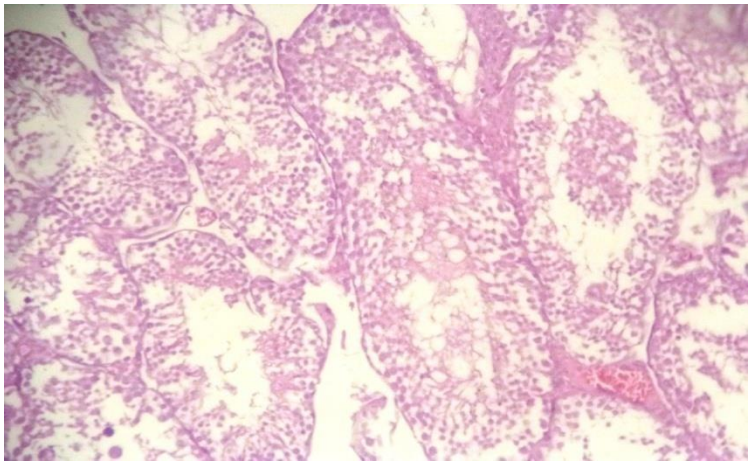


Figure (2): Histopathological section in testis of mouse treated with 300 mg/kg of *panax gensing* alcoholic extract daily, with 0.15 mg/kg of doxorubicin twice daily for five weeks showed the seminfrous tubules, showed mild changes when compared with other treated groups in which there is increase in number of spermatid and spermatozoa (H&E. X 100). .

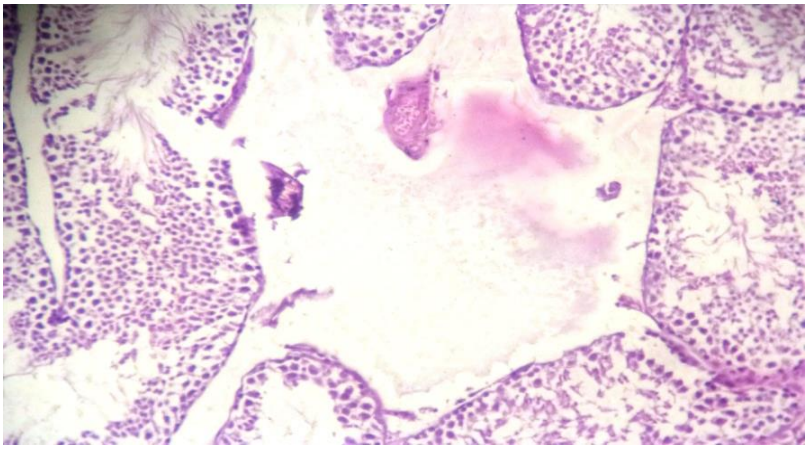
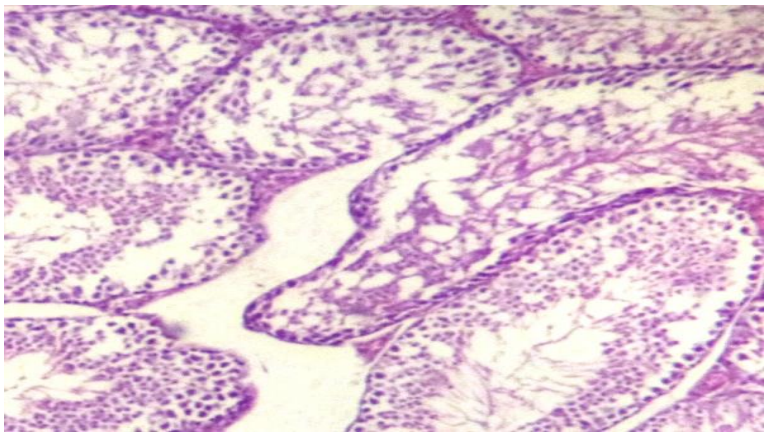


Figure (3): Histopathological section in testis of mouse treated with 0.4mg/kg of tamoxifen citrate drug daily, with 0.15 mg/kg of doxorubicin twice daily for five weeks, there is edema in the interstitial tissue and congestion of blood vessels (H&E. X 100). .



Figure(4): Histopathological section in testis of mouse preserved 150 mg/kg of panax gensing extract with 0.2mg/kg of tamoxifen citrate drug daily, with 0.15 mg/kg of doxorubicin twice daily for five weeks, there is a mild changes in the somniferous tubules in which there is very few number of spermatozoa when compared with negative control group, also there is congestion of blood vessels in the interstitial tissue (H&E. X 100).

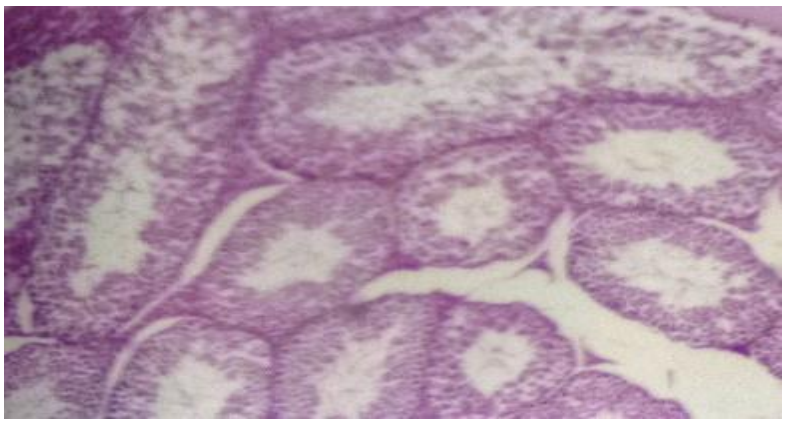


Figure (5): Histological sector in testis of normal mouse (H&E. X 100).

Discussion:

That results finding in table (1 and 2) for group treated with extract may be due to ability of ginseng extract to develop the sperms value and amount, these activities are commonly belong to ginsenosides, that is a main pharmacological dynamic constituents of ginseng, which are facilitate sperms capaciy and acrosome response by improving intracellular nitric oxide creation from endothelial tissue, and to enlarge smooth muscles sympathy to nitric oxide, and cyclic guanisine monophosphate which related to sperm function and improvement velocity of sperms (17). In addition the extract had stimulatory influence of ginseng seeds for DNA and proteins creation in testis (18), the increase of testosterone hormone in group treated with rxtract might be regarded to the main dynamic constituent (Ginsenoside) in *Panax* extract that enhance luteinizing hormone (LH) which is creation in Leydig tissue and produced by anterior pituitary lead to surge testosterone level, this result smaller to result reported by Kar WL, and Alice (17).

Histo Ginseng preserved mice was established an augmented degree of spermatogenesis by glial cells derived of neuro-trophics factors showed raise in Sertoli cell, and stimulation of cyclic adenosine mono phosphate (19), glial cells derived of neuro-trophics factors are a potential manager of persistence and cell outcome assessment of undifferentiated spermatozoa cell, and the cyclic of adenosine monophosphate is important for sperm development. animals with slight or no CAMP display particular inhibition of maturity of spermatid, that might be a probable reason of sterility (20).

The results of tamoxifen citrate drug in tables (1 and 2) and histological sections might be referred to that drug is non-steroidal Form one of anti-estrogen complex which (10), blocks estrogens receptor and have a mix antagonists and agonists action. Tamoxifen treatment led to abnormalities appeared after usage referred to the impairment arose through the epididymus transfer and the tamoxifen, straight or ultimately acted on epididymus functions this result agreement with result reported by Olson, *et al* (21), Tamoxifen treatment significantly increased motility of sperm, this referred to react the tamoxifen with negative feed-back hypothalamus and pituitary hormones, lead to increase gonadotropin hormones (GnRH) and luteal hormone discharge, which is encourages Leydig tissues of testis; lead to enhance testosterone hormone creation, thus improving spermatogenesis, an enhancement in fertility therefore Tamoxifen performed more alike an agonists than antagonists at the gonads axis, this results agreement with results reported by Corrada, *et al* (10).

In tables (1 and 2) and the histological section in group treated with mix extract with tamoxifen citrate appeared important improvement in sperm function test and turbidimetric analysis of sperms , this might be regarded to that extract decrease the side effect of tamoxifen drugs via its active components of ginseng panax extract as we mentioned above.

Conclusion:

From this study concluded the extract of *panax gensing* seeds play important role in increase fertility of male mice by enhance spermatogenesis and have the ability to decrease side effect of tamoxifen citrate.

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