



The Effect of An Aqueous Extract of Vitex Agnus-Castus Leaves on the Liver and Kidney in Female Rabbits

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Abstract

According to their accessibility and lower cost-effectiveness when compared to synthetic chemical treatments, medicinal wild plants and herbs have been used for ages as beneficial tools in the management of many diseases. The popular medicinal plant Vitex agnus-castus, which is indigenous to the Mediterranean region and widely distributed in Europe, Asia, and North Africa, is used to treat a number of conditions and diseases, including premenstrual syndrome, mastalgia, infertility, and menstrual cycle management. The current study examined how the aqueous extract of the vitex plant compared to clomiphene citrate in female rabbits. In this experiment, nine female rabbits were divided into three equal groups. Aqueous vitex plant extract was administered to the first group for 15 days, clomiphene citrate was administered to the second group for 5 days as aspect of an approved course of treatment, and distilled water was administered to the third group for 15 days as a control. To determine the impact of the aqueous extract of Vitex agnus castus on the functions of the female rabbits' body organs, the complete blood count (CBC) and GLU, UREA and CREA were calculated. In addition, the analyses of blood picture showed a significant disparity, enzymes; even histopathology results in compare with control which showed a significant decreasing in WBC count has observed in the vitex extract (F) group that was 4.7 for WBC and 4.45 for RBC while 9.8 for HGB. On the other hands, decreasing of WBC count observed significantly in the clomiphene citrate (C) group that was 3.9 for WBC and 5.3 for RBC while HGB was 6.4mg.

Keywords: herbal, vitex, extract, rabbits

Introduction

Throughout human history, herbal therapy has been widely used to treat a variety of ailments and diseases (1). The widespread use of herbal remedies including both culinary and medicinal reasons has contributed significantly to the growth of human civilization. Traditional medicine has utilized herbal remedies made from natural ingredients to treat and prevent a variety of human ailments, such as cardiovascular disorders (2). Many plant-derived natural compounds have previously been identified, and many of them have lately received approval for pharmaceutical application. They offer a great deal of potential to be utilized in treating a wide range of illnesses. As a result, therapeutical description of their effects, particularly the underlying intracellular mechanism involved, may help to better understand their roles and can be employed as a strategy for finding new applicable and mostly potent medications. However, several of their influence remain to be mechanistically described(3). In Western countries, the use of alternative medicines is on the ascent, with women making up the majority of those who utilize treatments for female reproductive wellness (4). For a diversity of female reproductive issues, the phytotherapeutic *Vitex agnus-castus* fruit (chaste tree, chasteberry; family: Verbenaceae) is routinely administered. As a result, *Vitex agnus-castus* is also used to treat a variety of illnesses, such as premenstrual syndrome (PMS) and related cyclic mastalgia, premenstrual dysphoric disorder (PMDD), existing problems with lactation, diminished fertility [5,] and concerns related to menopause (5), health symptoms linked to

menopause (6). Phytoestrogen-containing plants have earned the majority of interest to scientists among phytotherapeutic treatments, with other herbal ingredients historically often used alleviate menstrual cramps receiving surprisingly minimal attention (7). The reliability of long-term utilization among other phytoestrogens, in particular with respect to breast and endometrial tissue proliferation, has lately come under scrutiny; as a consequence, this research is based on nonestrogenic substances routinely stipulated for menopausal symptoms and adopted a new approach (8)

Materials and Methods

Experimental design: Initially, the current experiment was prepared and designed by using three groups of adult female rabbits, each group consisting of three females rabbits (n=3), Comparison among groups of treatments with control, all parameters was done by one-way analysis of variance using SPSS 16 statistical software. Rabbits with weight 3 kg hospitalized for 10 days for adaptation with same environmental conditions, then, the first group orally dosage with vitex (aqueous extract) for 15 days. Consequently, the second group treated with approval drug clomiphene citrate for 5 days (according to course timing of drug) to compare therapeutic effect with aqueous extract of vitex. The third group treated with DW as a vehicle control group. All animals kept under similar conditions, food and drinks (Fig 1).

Prepare the extract:

Following the drying and weighting of 200 mg/kg of vitex leaves with a sensitive scale, the leaves were crushed in a mortar, 600 ml of distilled water was added for soaking, the beaker was placed on a stirring machine for 48 hours, the plankton (insolvable leave parts) were removed with filter paper, the extract was placed in an oven at 50 ° C for evaporation and extraction of the active substance for a week, and the extract was eventually obtained The fertility-increasing medication clomiphene citrate is used as a positive control.

- **Samples collection:**

- a. **Blood sample collection:**

After the trial period has ended (15 days). Blood samples have taken gently with a 19-21-gauge needle to prevent the heart from collapsing after the animals have been fasted for 12 hours and anesthetized with ether for several minutes. Around 3 ml of blood extracted, preferably from the ventricle, which can be accessible via the left side of the chest, through the diaphragm, from the top of the sternum, or via a thoracotomy. For a later CBC test, the blood transferred to 10 mL sterile tubes containing heparin.

- b. **Histological Examination:**

After sacrificing the animals, the liver, kidney collected and embedded with 10% buffered formaldehyde, then, slides were prepared and stained with H&E stains and examined histopathology changes under light microscope.

Results

The effect of vitex leaves extract on the blood parameters: -

Blood parameters have mentioned in Table (1) showed Vitex extract (F) and clomiphene citrate (C) resulted in a significant decrease in (RBC), (WBC) and (HGB), in comparison with control (M), While a significant decreasing in WBC count has observed in the vitex extract (F) group. On the other hands, decreasing of WBC count observed significantly in the clomiphene citrate (C) in comparison to the control group as shown in the table below.

Impacts of clomiphene citrate (C) (50 mg per kg). The outcome was that the clomiphene citrate treatment group (C) observed a rise in creatinine levels of 1.29 mg/dl at the same time as glucose levels showed a decrease of 2.9 mg/dl and an increase of 15.6 mg/dl In the vitex extract (F), the glucose level (GLU) is 2.8 mg/dl lower but the urea level (UREA) is 16.5 mg/dl higher ($p > 0.05$). There is also a 0.43 mg/dl increase in creatinine levels. The table 2 below shows the comparison with the control group.

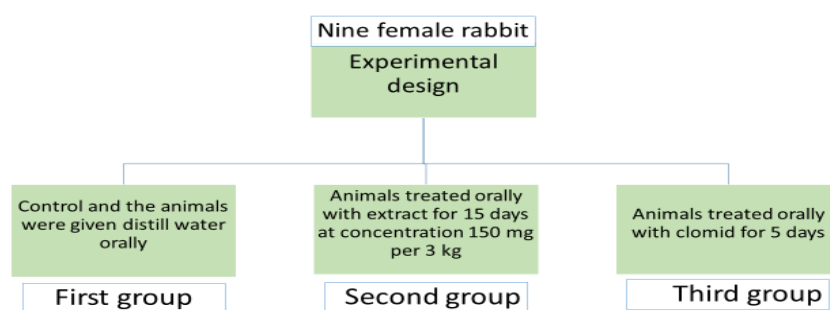


Figure (1) shows the design of the experiment

Table 1. Shows the blood parameters of all groups of experiment.

Groups of treatment	WBC (10*3)	RBC (10*6)	HGB%
Control (M)	6.63 ±12	5.9±1	12.4±08
Vitex extract (F)	4.7±02*	4.45±04*	9.8±06*
Clomiphene citrate (C)	3.9±05**	5.3±02	6.4±02**

Notes: Data expressed as Mean ± SE, * = $P < 0.05$, **= $P \leq 0.01$ and ***= $P \leq 0.001$.

Table 2. Shows the testing enzyme of blood for all groups of experiment.

Treatment groups	Glucose (mg/dl)	UREA (mg/dl)	Creatinine (mg/dl)
Control (M)	3.9 ± 12	14.7 ± 06	0.35 ± 08
Vitex extract (F)	2.8 ± 05*	16.5 ± 08	1.29 ± 04***
Clomiphene citrate (C)	2.9 ± 06*	15.6 ± 021	0.43 ± 04*

Notes: Data expressed as Mean ± SE, * = $P < 0.05$, **= $P \leq 0.01$ and ***= $P \leq 0.001$.

Histopathological finding: By comparing between the groups, there are many visible changes in the liver and kidneys, where the occurrence was observing in clomiphene citrate treated group in liver exerting sinusoidal dilatation with congested central vein, hyperplasia of bile duct, leukocytic infiltration hepatocytes vacuolation. Moreover, in kidney show renal tubules damage and showed diffuse leukocytic infiltration, significant renal epithelial lining cytoplasmic vacuolation. While in liver of group treated with Vitex extract showed, sever vascular dilatation, significant hepatic tissue damage represented by fibrosis (fibrotic nodule), and hepatocytes damage with significant necrosis. mild RBCs extravasation .and in kidney in group treated with Vitex extract showed moderate renal tubules damage with significant necrosis, inflammatory cells infiltration, significant vacuolation of epithelial lining, mild RBCs extravasation MNCs infiltration.

Discussion:

The results of the current study show that the extract herbal vitex leaves toxic effect after treatment with vitex extract dose 150 mg per female rabbit weighing 3 kg, for 15 days. By consequence, the results of this group exhibits a significant effect with strong decrease in the percentage of WBC by 4.7 compared to the normal level of 6.63, as well as a decrease in both RBC and HGB, as seen in table 1, our results are nearly close to some studies with difference of courses of treatment (9). Some preclinical findings indicate that the kidney can release erythropoietin, a humoral component responsible for the control of red cell generation in rabbits(10). when the

researchers utilize aqueous vitex leaf extract supplemented at 7.5 g/kg per kilogram in the diet of rabbits which increased their reproductive and productive performance in mice. The above findings corroborate with (11) claim that vitex extract had no noticeable impact on white blood cells when compared to the control group. Interestingly, It has been demonstrated that the flavonoids in this plant can reduce the amount of red blood cells, packed cell volume, and hemoglobin, which may explain why some blood parameters have decreased(12) Alcoholic extract, however, contained alkaloids, saponins, anthraquinones, flavonoids, and terpenoids in vitex leaves(13).A significant increase in the level of urea and creatinine observed and decreasing in the level of glucose in the group of rabbits that used extract herbal vitex leaves on them. On the other hands, the effect of clomiphene citrate has given different effects from the extract which observed by decreasing level of glucose and raising level of urea, creatinine in slight proportions, as see in table 2, results of current study close other research applied in Iraq (14). In addition, the liver showed sever vascular dilatation, significant hepatic tissue damage represented by fibrosis (fibrotic nodule) Figure (7). Sever hepatocytes damage with significant necrosis, mild RBCs extravasation with fibrosis figure (8) the similar changes noticed by researchers when they treated the rats with vitex(14). These findings therefore represent a step toward identifying a novel herb for use in both conventional and alternative medicine.

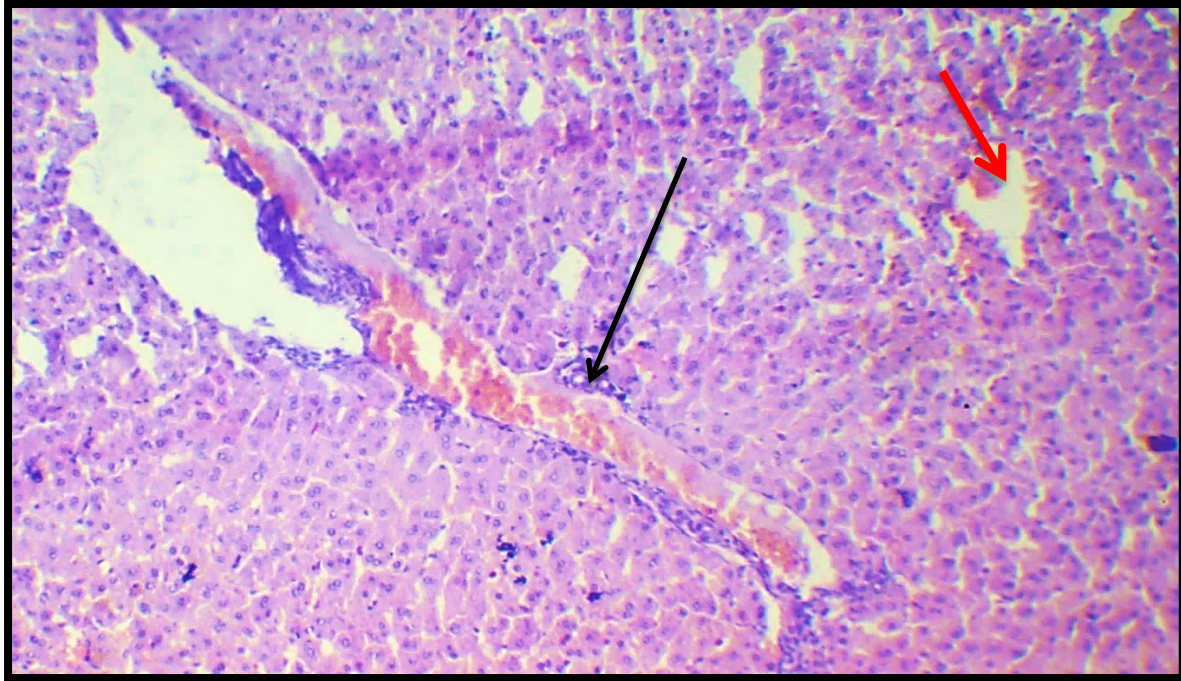


Figure (2) Photomicrograph of liver effected by clomiphene citrate, which revealed the characteristic sinusoidal dilatation with congestion (black arrow), congested central vein (red arrow). (H and E ,10X).

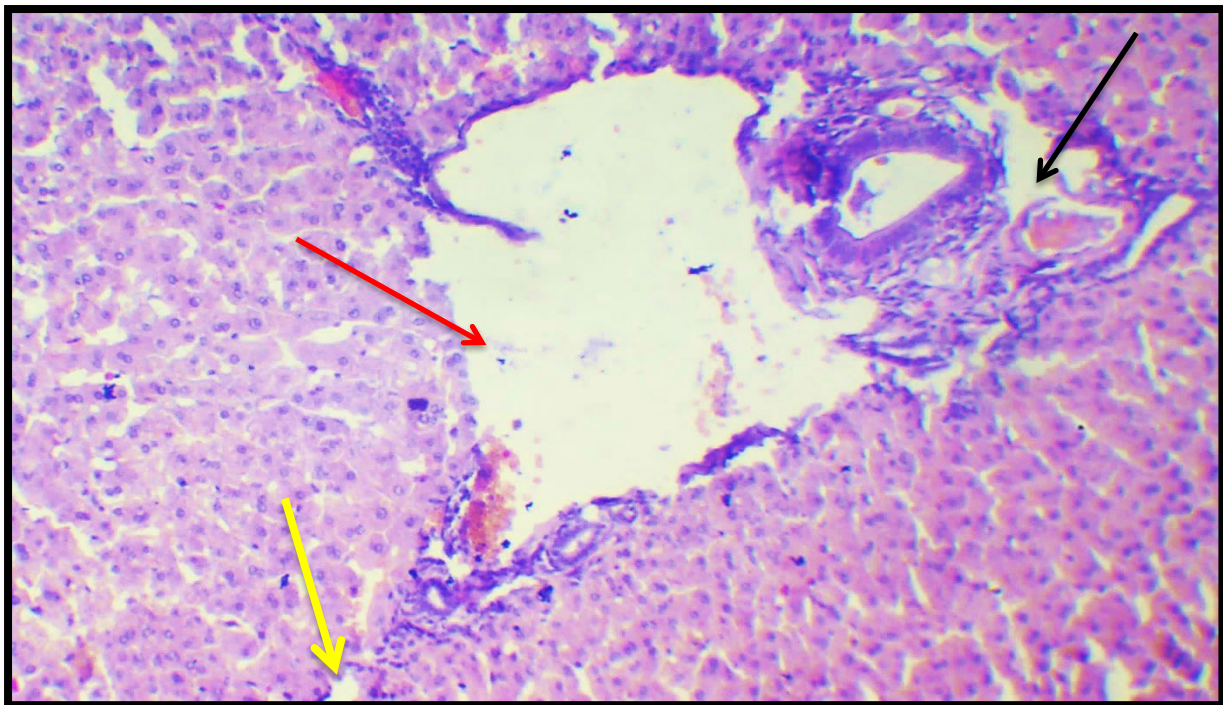


Figure (3) Photomicrograph of liver effected by clomiphene citrate, which revealed significant hyperplasia of bile duct (black arrow), sever enlarged and congested portal vein (red arrow) and leukocytic infiltration (yellow arrow). (H and E ,10X).

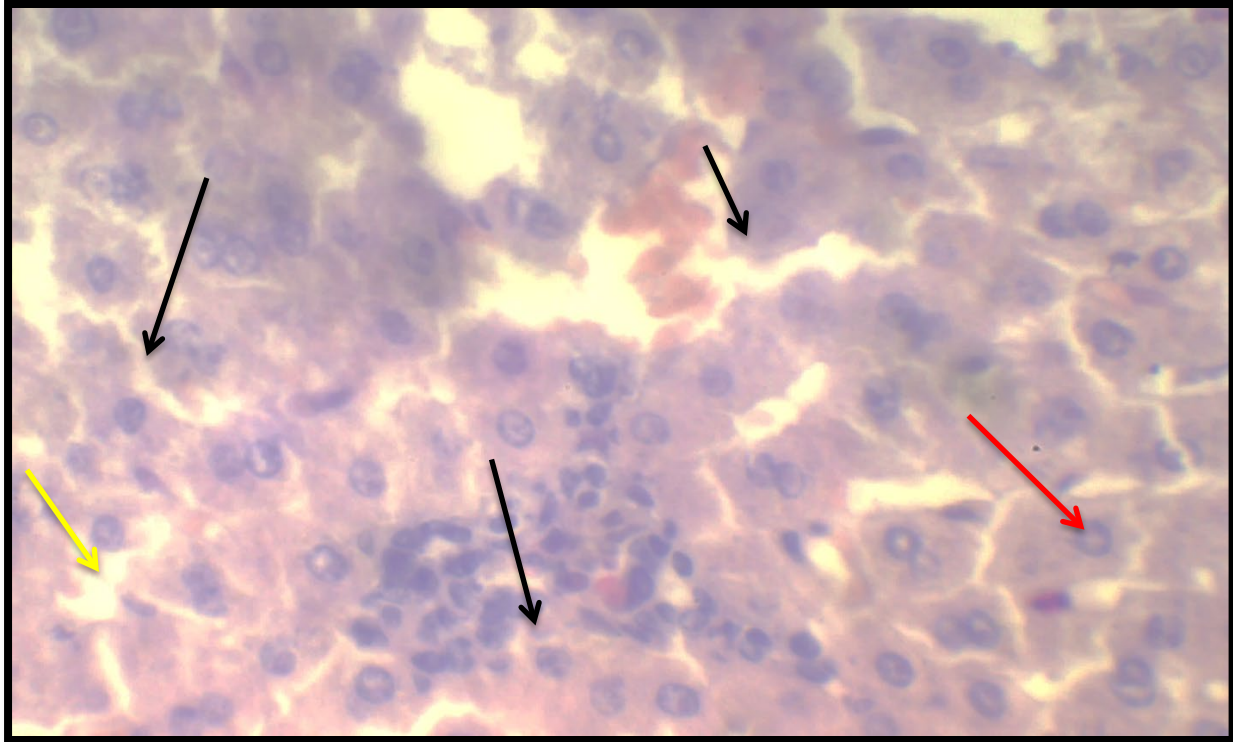


Figure (4) Photomicrograph of clomiphene citrate treated group liver revealed focal leukocytic infiltrates (black arrow), significant cytoplasmic hepatocytes vacuolation (red arrow) with sinusoidal congestion (yellow arrow). (H and E ,40X).

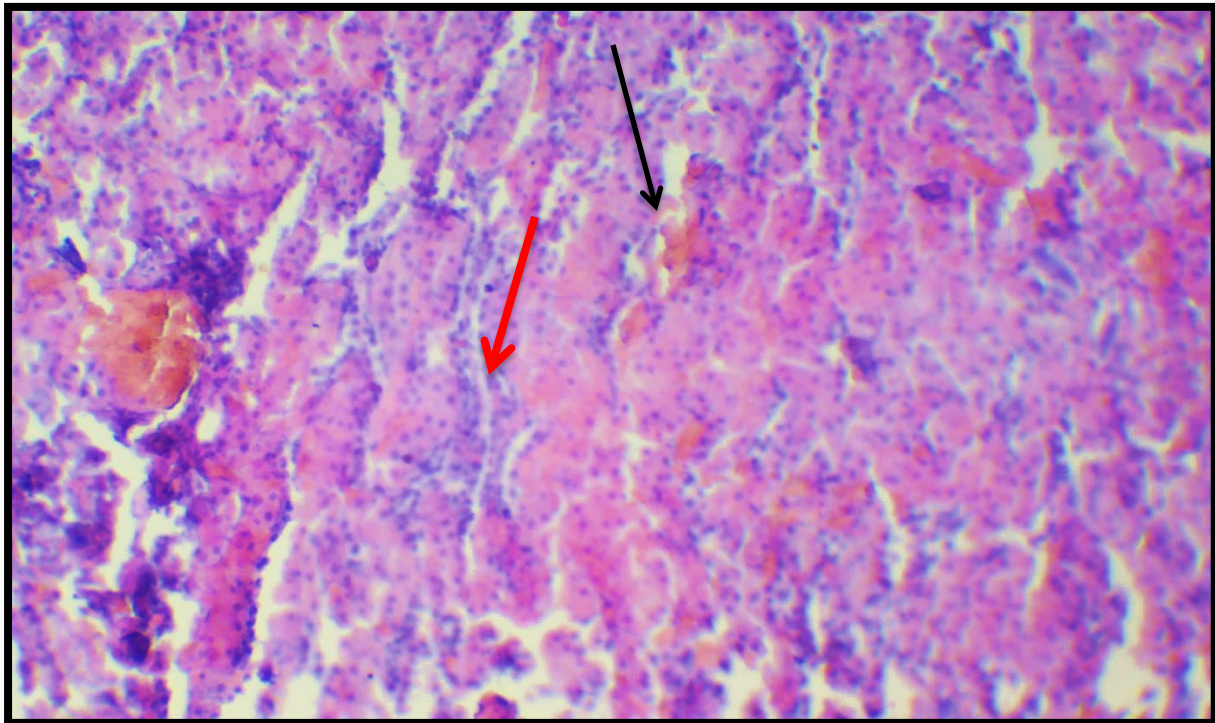


Figure (5) Photomicrograph of kidney of a rabbit treated with clomiphene citrate showed severe vascular congestion (black arrow), significant renal tubules damage (red arrow).(H and E)

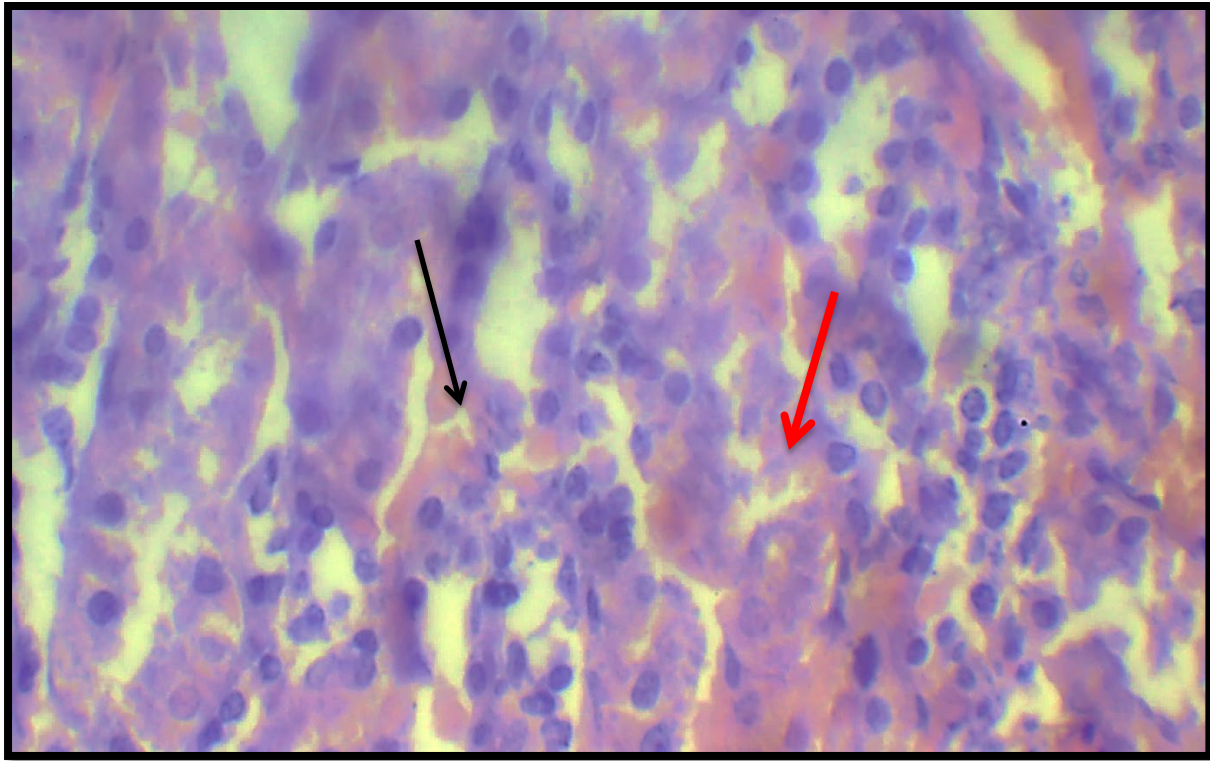


Figure (6) Photomicrograph of kidney of a rabbit treated with clomiphene citrate showed diffuse leukocytic infiltration (black arrow), significant renal epithelial lining cytoplasmic vacuolation (red arrow). (H and E, 40X).

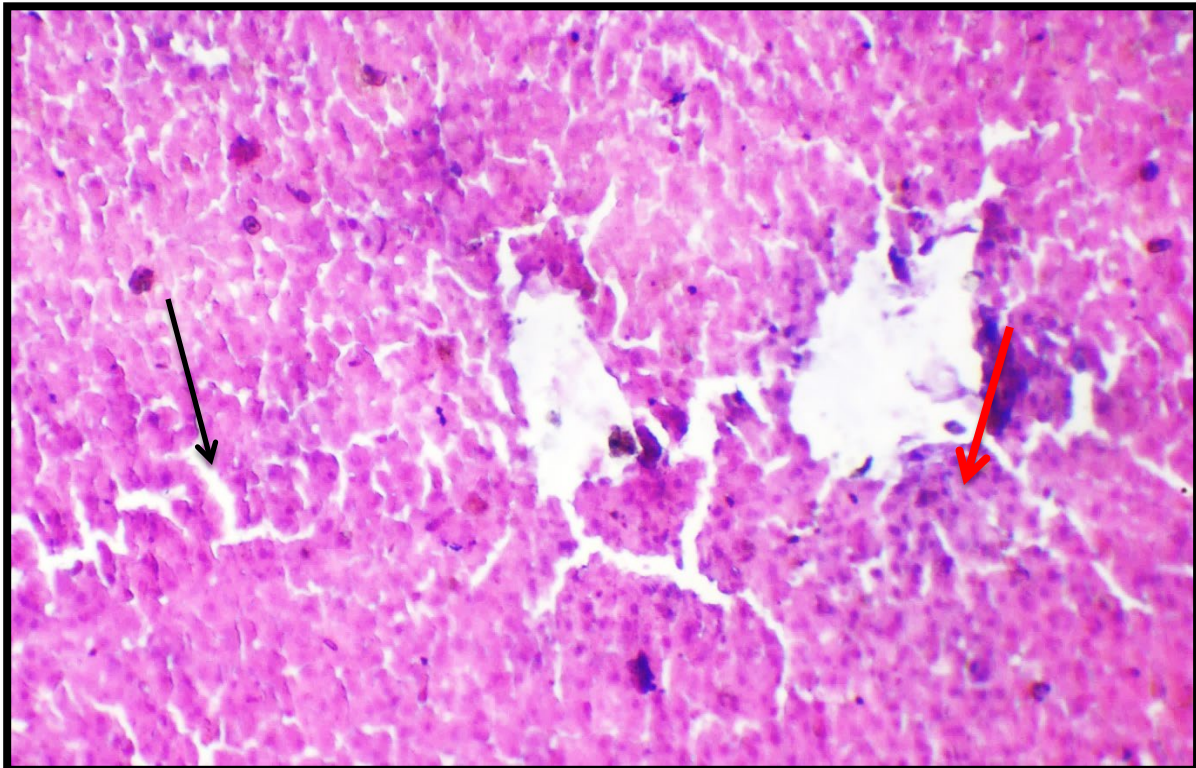


Figure (7) Photomicrograph of liver of a rabbit treated with Vitex extract showed sever vascular dilatation (black arrow) , significant hepatic tissue damage represented by fibrosis (fibrotic nodule) (red arrow).(H and E ,10X).

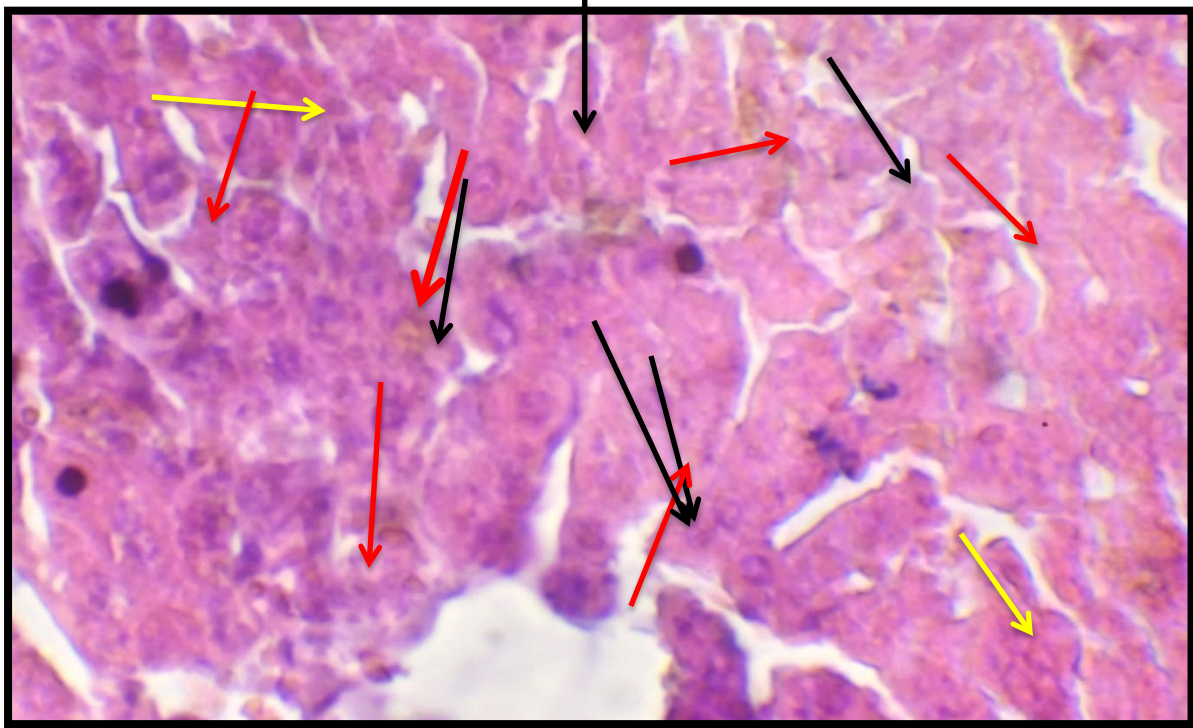


Figure (8) Photomicrograph of liver of a rabbit treated with Vitex extract showed sever hepatocytes damage with significant necrosis (black arrow), mild RBCs extravasation (red arrow) with fibrosis (yellow arrow). (H and E, 10X).

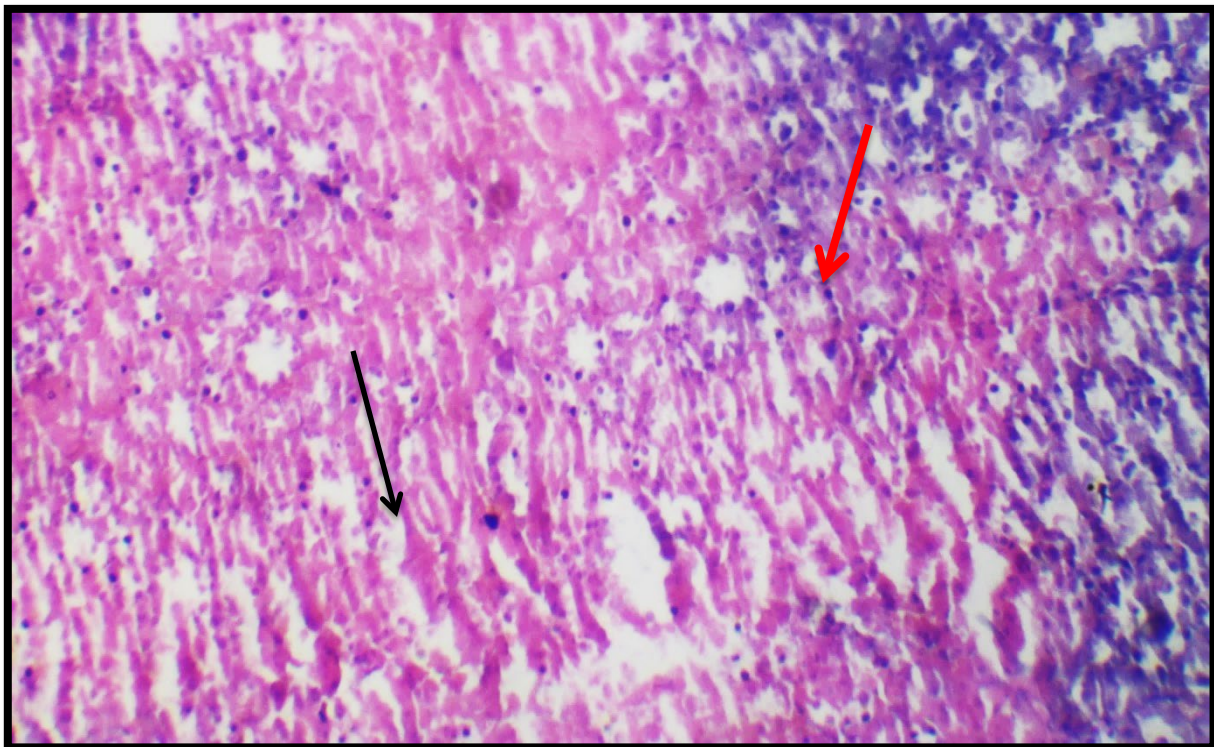


Figure (9) Photomicrograph of kidney of a rabbit treated with Vitex extract showed moderate renal tubules damage with significant necrosis (black arrow), inflammatory cells infiltration (red arrow). (H and E, 10X).

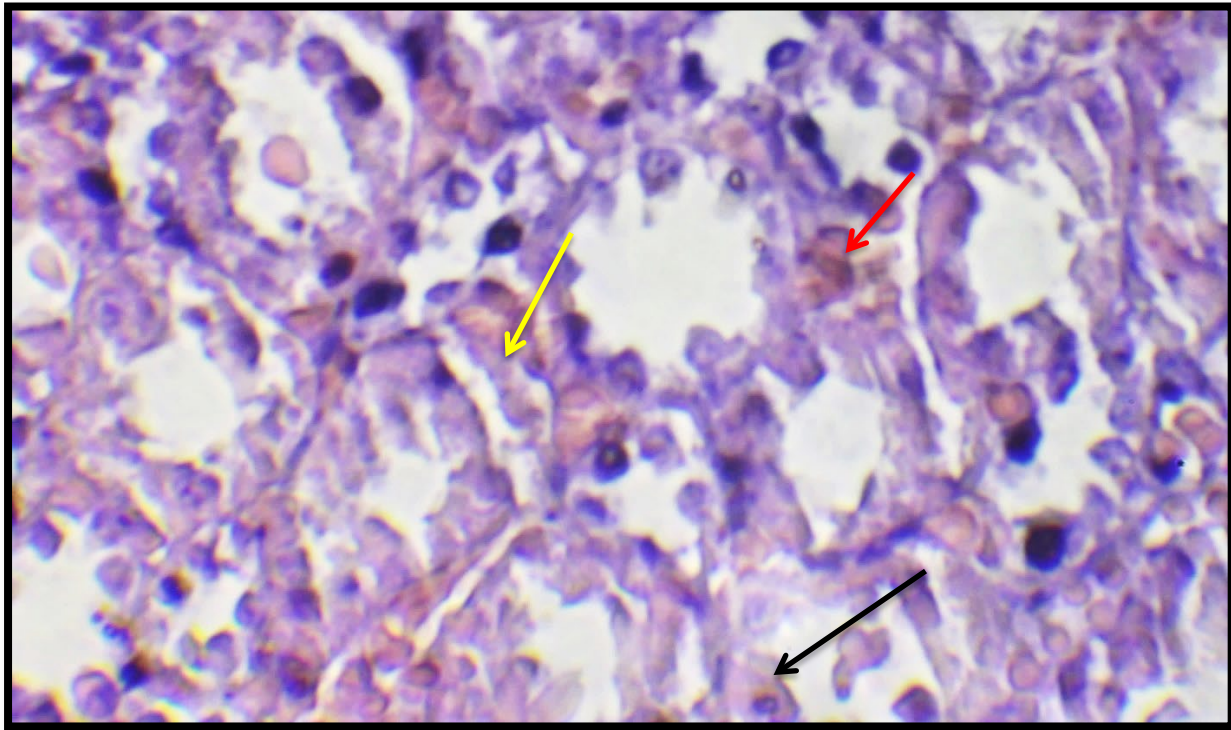


Figure (10) Photomicrograph of kidney of a rabbit treated with Vitex extract showed significant vacuolation of epithelial lining (black arrow), mild RBCs extravasation (red arrow) MNCs infiltration (yellow arrow). (H and E).

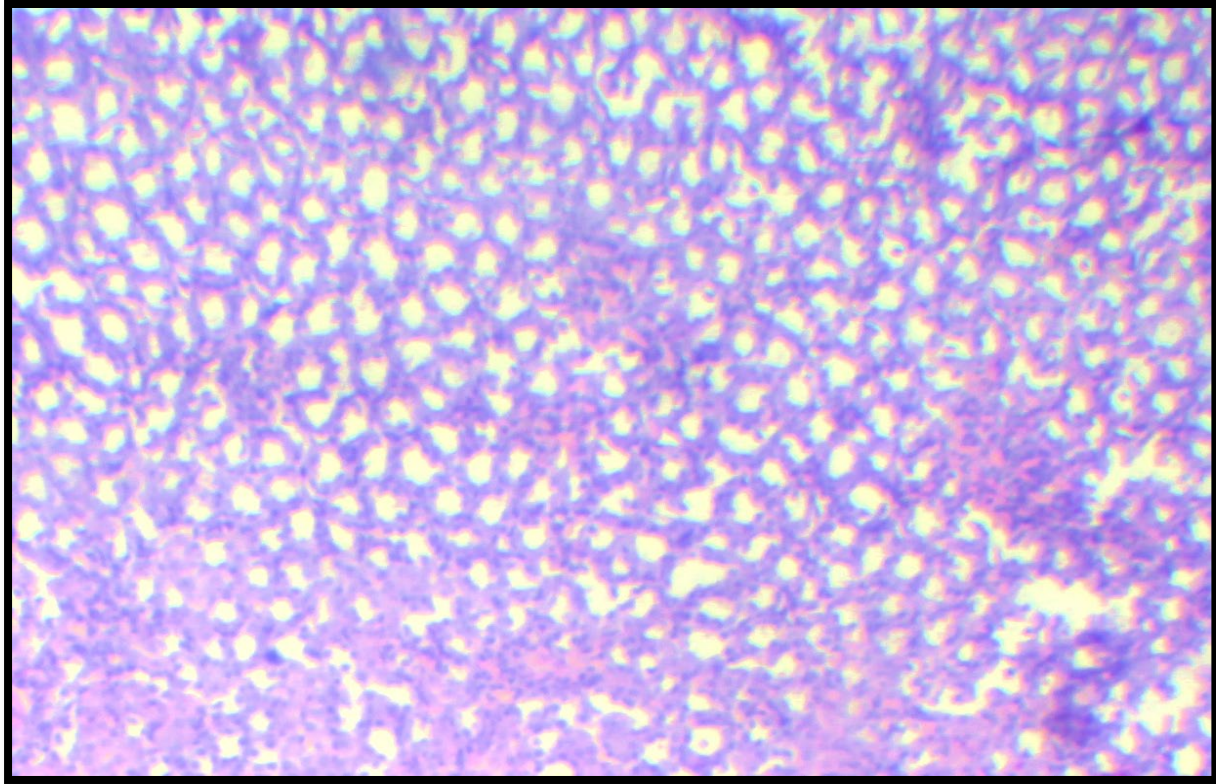


Figure (11) histological section of a kidney of control group animal showed normal renal tubules. (H and E,10X).

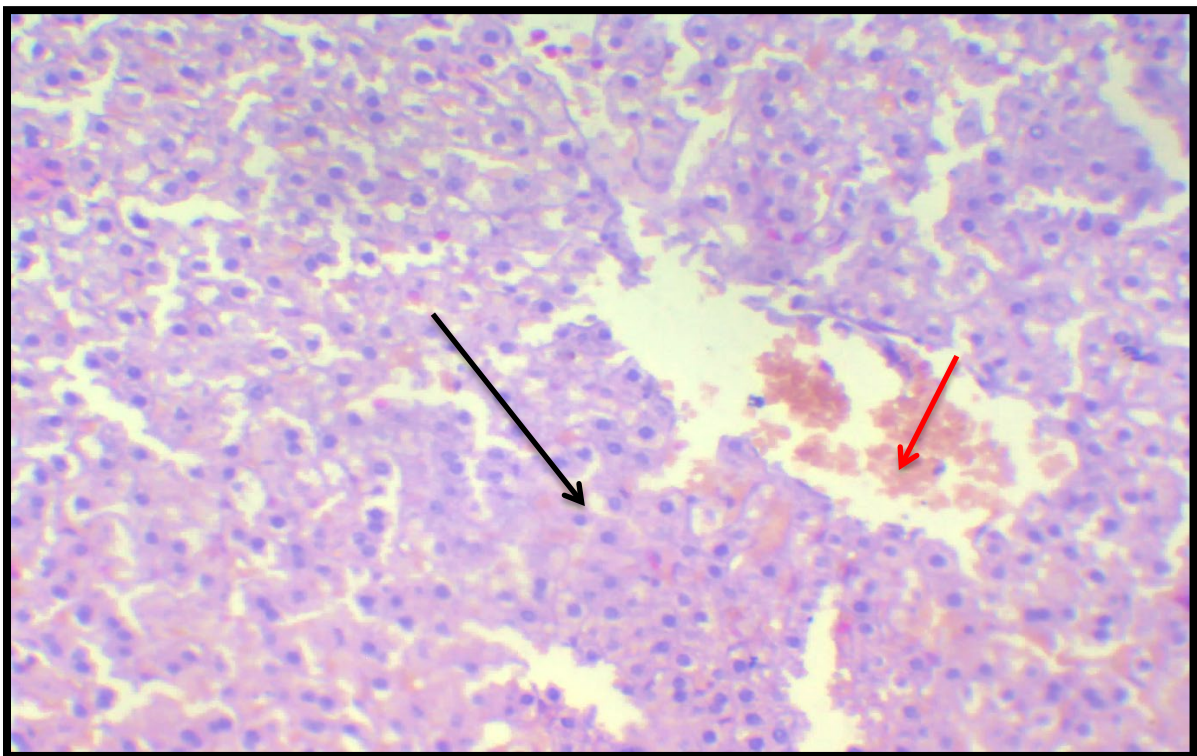


Figure (12) histological section of a liver of control group animal showed normal hepatocytes

arrangements with mild degenerative changes (black arrow), blood vessel congestion (red arrow). (H and E, 10X)

conflict of Interest

The author(s) declared that there is no conflict of interest.

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تأثير المستخلص المائي لأوراق Vitex Agnus-Castus على الكبد والكلى في إناث الأرانب

نظرا لإمكانية الوصول إليها وانخفاض فعاليتها من حيث التكلفة عند مقارنتها بالعلاجات الكيميائية الاصطناعية، فقد تم استخدام الأعشاب والأعشاب البرية الطبية على مر العصور كأدوات مفيدة في إدارة العديد من الأمراض. منها لنبات الطبي كف مريم، وهو نبات أصلي في منطقة البحر الأبيض المتوسط وينتشر على نطاق واسع في أوروبا وآسيا وشمال إفريقيا، لعلاج عدد من الحالات والأمراض، بما في ذلك متلازمة ما قبل الحيض، وألم الثدي، والعقم، ودورة الطمث. بحثت الدراسة الحالية كيفية مقارنة المستخلص المائي لنبات فيتكس مع سترات الكلوميدين في إناث الأرانب، حيث تم تقسيم تسع إناث الأرانب إلى ثلاث مجموعات متساوية في هذه التجربة. وتم إعطاء مستخلص النبات المائي للمجموعة الأولى لمدة 15 يوماً، وإعطاء سترات الكلوميدين للمجموعة الثانية لمدة 5 أيام كجزء من مسار العلاج المعتمد، وأخيراً تم إعطاء الماء المقطر للمجموعة الثالثة لمدة 15 يوماً كمجموعة سيطرة. لتحديد تأثير المستخلص المائي لـ كف مريم على وظائف أعضاء جسم الأرانب الأنثوية وذلك بحساب تعداد الدم الكامل و الكلوكوز و اليوريا و أظهرت تحليلات صورة الدم تبايناً كبيراً حيث سجلت النتائج انخفاضاً كبيراً في عدد كرات الدم البيضاء وبفارق معنوي و أيضاً لوحظ تبايناً في الهيموجلوبين و وكریات الدم الحمراء و حتى نتائج التشريح المرضي مقارنة مع مجموعة التحكم