

**STUDY THE EFFECT OF AQUEOUS EXTRACT OF *Lawsonia Inermis* ON SOME BLOOD PARAMETER AND HISTOPATHOLOGICAL CHANGE IN THE OVARY AND UTERUS OF FEMALES RABBITS**

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**Keyword:** Rabbit, WBC, Triglyceride.

**ABSTRACT**

The present study was under taken the effect of *Lawsonia Inermis*(Henna) extract on some hematological parameters, biochemical parameters and histopathological Changes in ovary and uterus of females rabbit.

Eighteen female rabbits were divided in to three groups (6 rabbit for each group). The first group treated with normal saline orally daily for 30 days (control), the second group treated with(500mg/kg B.W) of *Lawsonia Inermis* orally for 30 days, and the third group treated with (1000mg/kg B.W) orally for 30 days. *Lawsonia Inermis* extract showed significant decreased ( $p>0.05$ ) in RBC, WBC and Hb in dose (500&1000mg/kg B.W) when compared with control group. Total cholesterol and triglyceride decreased significantly in second and third group compared with control group. In addition to that the significant pathological changes were observed in the ovary and uterus which suggestive hyperplasia or fibrosis changes.

**INTRODUCTION**

*Lawsonia inermis* L. is a much branched glabrous shrub or small tree, cultivated for its leaves although stem bark, roots, flowers and seeds have also been used in traditional medicine .The henna is composed of colouring Of material (Lawsonia), 2-hydroxy-1:4naphthaquinon and other component carbohydrates, proteins, flavonoids, tannins and phenolic compounds, alkaloids, terpenoids, quinones, coumarins, xanthones and fatty acids ( 1,2). The *Lawsonia Inermis* (henna) is belong to family Lythraceae. (2). on other hand, *Lawsonia Inermis* is important medicinal herb has biological activities and pharmacological functions(3). Also it has been used in

cosmetic and medicine(1). In addition(4) reported that *Lawsonia Inermis* is against hyperlipidemic and hyperglycaemic activities. Even though anti-inflammatory, analgesic and antipyretic effects of henna have been shown, it may cause severe side-effects in some cases. For this reason, especially, in the regions where G6PD enzyme deficiency is common, people should be informed about the side-effects of topical henna application and clinicians should be aware of these manifestations(5). In addition, there are many experimental studies related to toxicity and adverse effects of henna on circulation and on the liver(6).. The purpose at this study was to investigate the effect of aqueous extract *Lawsonia Inermis* on some hematological and biochemical parameters such as (RBC,WBC, Hb, triglyceride and cholesterol) histopathological changing in ovary of female rabbits.

## MATERIAL AND METHODS

Leaves of *Lawsonia Inermis* were purchased from local market in Basra province /Iraq. The leaves part of henna was grounded mechanically using blender for (4 min), then dried at room temperature for 2 day under the shade. Eighteen female domestic rabbits were brought from the local market/Basra weighting (300-400) grams and age between (8-9) weeks. The rabbit were kept under observation in animal house of the college of veterinary medicine/University of Basra for four weeks. The animals were offered balanced rabbits diet that consist of green leaves, fodder & water *ad libitum*.

The animals were divided into three groups (6 rabbit for each group):

**Group 1:** rabbit were served as control which was treated with (2ml) of normal saline orally daily for 30 days.

**Group2:** The animals were treated with (500 mg/kg B.w) aqueous extra of *Lawsonia Inerims* orally daily for 30 days

**Group3:** The animal were treated with (1000gm/kg B.W) of aqueous extract of *Lawsonia Interims* orally for 30 days.

**Blood collection :** At end of experiment the blood Sample were collected from heart after sacrificed by using syringe ( 1ml ) of blood samples were collected into tubes

with anticoagulant ( EDTA ) for hematological study such as RBC and WBC count were determined by using hemocytometer method (7). The hemoglobin ( Hb ) concentration was determined by using sahli method (8).

**Biochemical measurement:** the total cholesterol and triglyceride were determined by using commercial kits (spinreact/ CHOD- POD, Spain).

**Histological techniques:** After the blood Collection, the organ samples removed & fixed in 10% buffered formalin, dehydrated progressively in increased ethanol concentration treated with xylene and embedded in paraffin. Five micron thickness Section cutting routinely prepared using a microtome and then tissue were mounted on glass slides and stained with Heamatoxylin & Eosine stain (9).

## RESULTS AND DISCUSSION

The results showed that treated group with aqueous extract of *Lawsonia Inermis* at dose (1000 mg/kg B.W) daily for 30 days was more significant than in case of the (500mg/kg BW) in red blood cell count, white blood cells and hemoglobin concentration compared with control group (table 1).

**Table 1:** Effect of *Lawsonia Inermis* on hematology parameter of female rabbits.

Group	RBCx10 <sup>6</sup>	Hb	WBCx10 <sup>3</sup>
Control(Normal saline	5.711±0.332 <sup>c</sup>	11.376±0.291 <sup>c</sup>	5.028 ± 0.156 <sup>c</sup>
<i>Lawsonia Inermis</i> (500mg/kg)	5.893±0.284 <sup>b</sup>	11.30±0.728 <sup>b</sup>	35.766±1.434 <sup>b</sup>
<i>Lawsonia Inermis</i> (1000mg/kg)	4.481±0.247 <sup>a</sup>	8.450±0.556 <sup>a</sup>	26.133±0.969 <sup>a</sup>
significance	*	*	*

Values are expressed as mean± stander deviation .small letters denote differences between groups, (p≤ 0.05) compared with control.

The result are in agreement with(10)are found That *Lawsonia Inermis* causes oxidant to red blood cell , because Chemical ingredient henna is Lawson (2-hydroxy-1,4naphtho-quinone). Its structure and redox potential is similar to 1, 4 naphthoquinone, a metabolite at of naphthalene material.

On the other hand, white blood cells was significant decreased 500 and1000mg/kg B.W compared with control group. This result may be related to exist some kind of inhibition for the active ingredients of henna heamatopoiesis (11) said that saponin also has haemolytic effect. . In addition (12) shows that extract in higher dose level has deleterious effect on white blood cell but there is significant increase in hemoglobin.

The result of total cholesterol and triglyceride in group of animal treated with aqoues extract of *lawsonia inermis* decreased significantly ( $p \geq 0.05$ ) in comparison with control group (table 2).

Table 2: effect of *Lawsonia Inermis* leave after 30 days of administration on total cholesterol and triglyceride level in female rabbits.

group	Total cholesterol Mg/dl	Triglyceride Mg/dl
Control(normal saline)	109.2 ± 4096 <sup>a</sup>	108.233± 0.94 <sup>a</sup>
Lawsonia Inermis(500mg/kg B.W)	86.8±2.815 <sup>b</sup>	96.6± 2.361 <sup>b</sup>
Lawsonia Inermis(1000 mg/kg B.W)	102.86± 8.724 <sup>c</sup>	88.20 ± 5.263 <sup>c</sup>
significance	*	*

Values are expressed as mean± stander deviation .small letters denote differences between groups, ( $p \leq 0.05$ ) compared with control.

These finding similar to that of (13) who demonstrated that *L. inermis* extract showed the anti-hyperglycemic effect which may be due to potentiation of insulin

release from  $\beta$ -cell of islets, or as a result of enhanced transport of blood glucose to muscles and adipose tissues (14).

Histological section of the ovary derived from rabbits with dose Of 500mg/kg B.W showed hypertrophy of epithelial cell of ovary and immateration of ovarian follicle (figure 2,3) compared with control(figure 1).

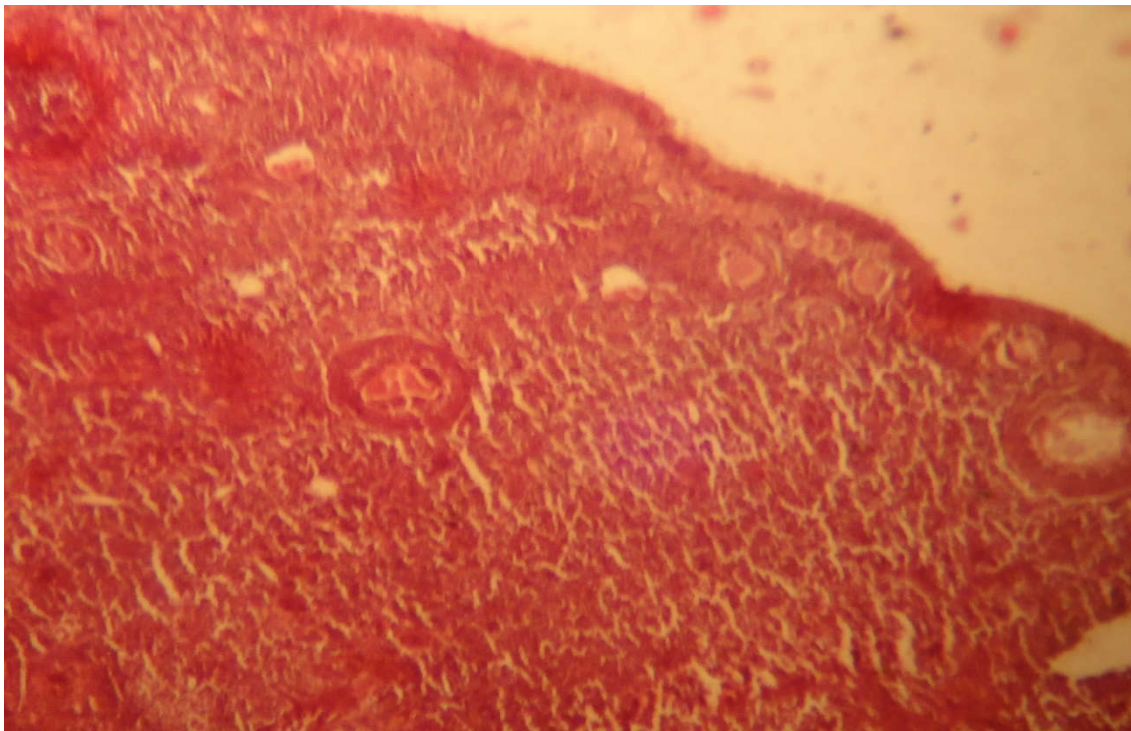


Figure 1: Section of ovary of control female rabbit shows normal ovarian tissue.H&EstainX400

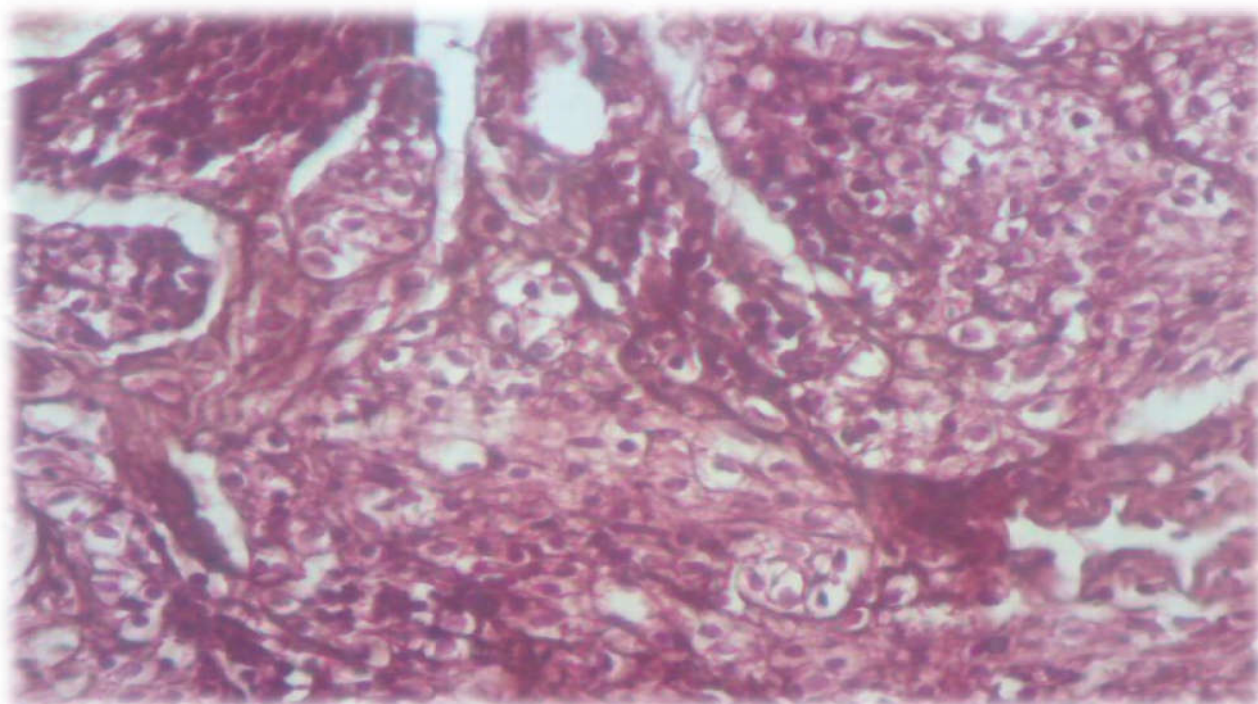


Figure 2: ovary of rabbit treated with 500 mg/Kg *lawsonia inermis* stained with (H&E) X40. Hypertrophy of ovary epithelial cell with necrosis

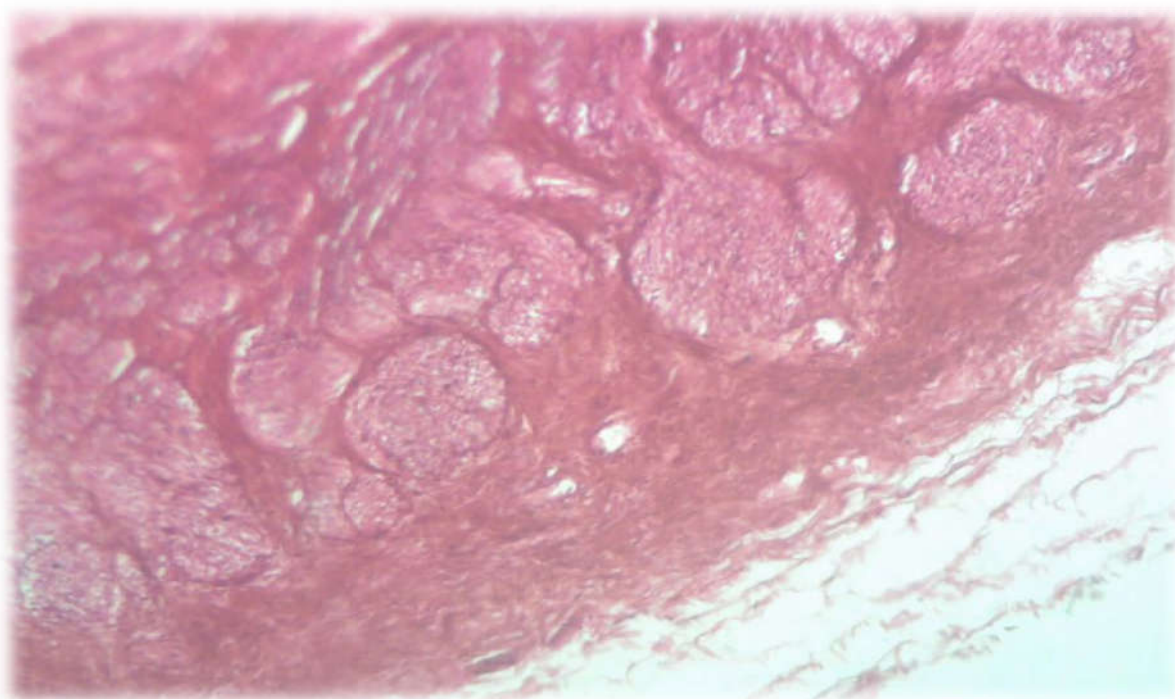


Figure 3: ovary of rabbit ovary of rabbit treated with 500mg/Kg *lawsonia inermis* stained with (H&E) 40X.narrowing in the lumen of follicle and degenerative change.

Sever necrosis, hemorrhage area with hyperplasia of ovarian gland observed in the tissue of ovary treated at dose 1000mg/kg B.W (figure 4, 5) compared with control (figure 1). Some studies find that some degree of toxicity and adverse effect on circulation at higher dose, our result is in agreement with finding of (15) who detected distortion in the normal histology of the ovary, preventing the formation of follicular cavity and arresting Oocytes maturation.

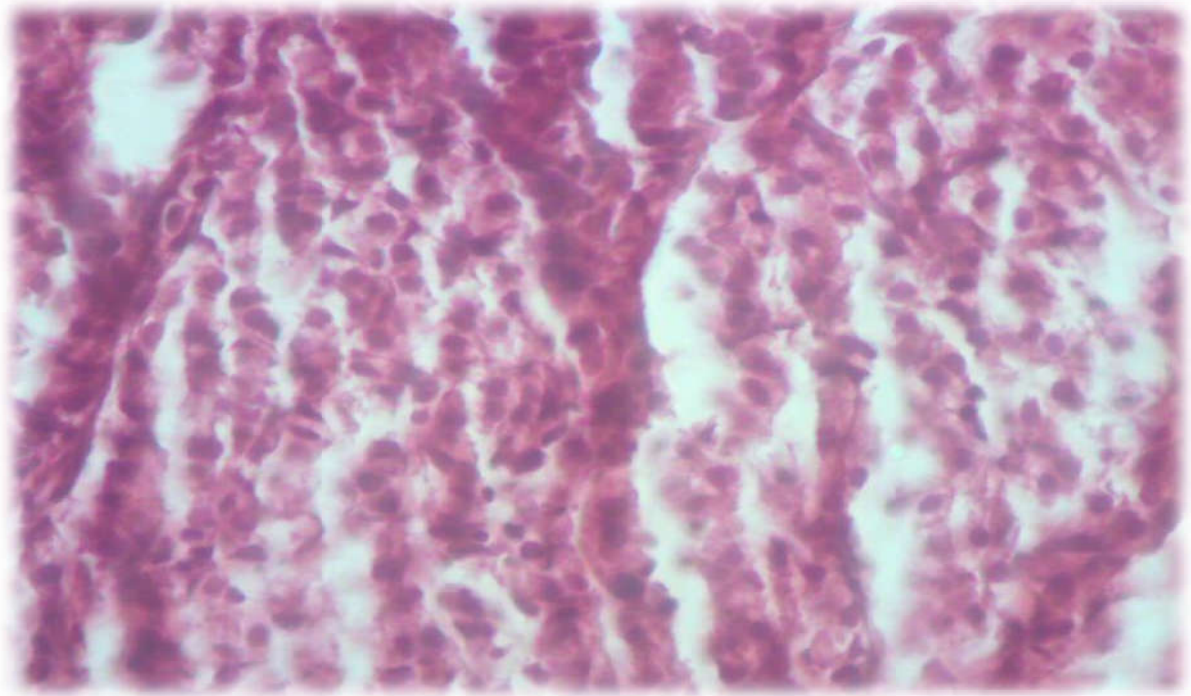


Figure 4: ovary of rabbit ovary of rabbit treated with 1000mg/Kg *lawsonia inermis* stained with (H&E) 40X.hyperplasia and interstitial fibrosis of overian gland.

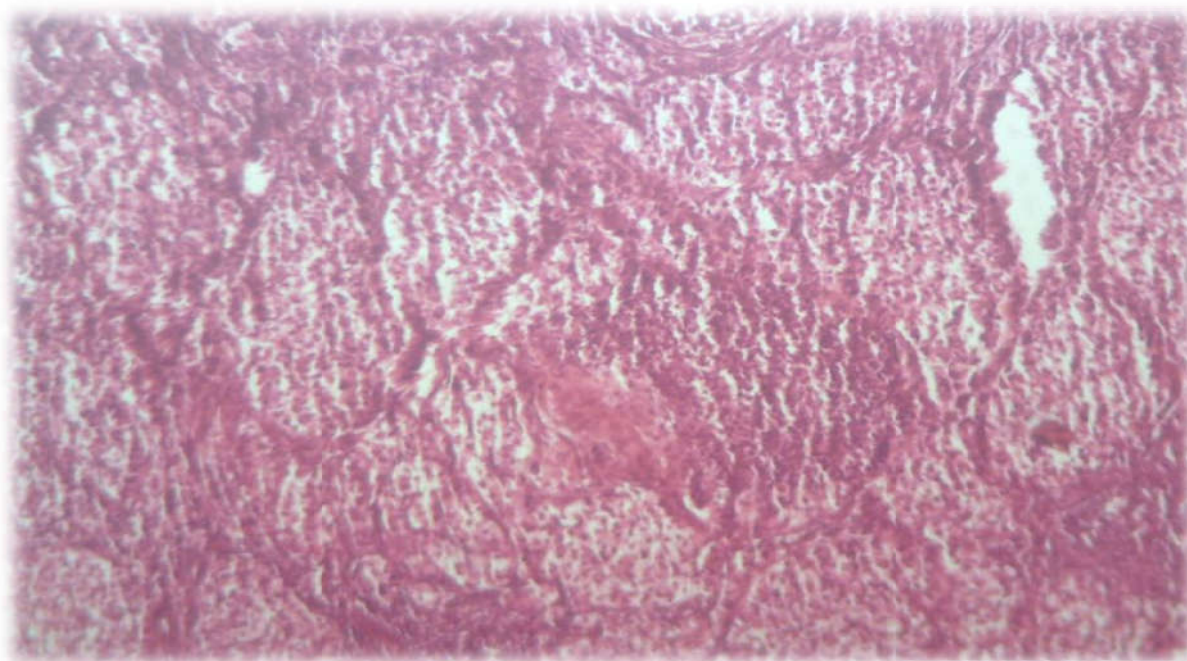


Figure5: ovary of rabbit ovary of rabbit treated with 1000mg/Kg *lawsonia inermis* stained with (H&E) 40X.defuse hemorrhage and sever necrosis of ovary.

On the other hand, the uterus showed clear hyperplasia of epithelial cell. In uterine gland of female rabbits administrated (500 mg/kg b.w)of *l. inermis* as compared with control animal(figure,6,7).While at dose (1000 mg /kg b.w) of *l. inermis* for 30 days shows atrophid ,fibrosis and decrease in the number of uterine gland(figure,6,8).These results agreement with(16 and 17) who noted *Lawsonia inermis* extract was given to female caused loss of implantation sites and Phytochemical screening of the extracts showed the presence of glycosides, phytosterol, steroids, saponins, tannins and flavonoids . It is suggested to screen specific phytochemical components from *Lawsonia inermis* which particularly induces loss of implantation sites. Also Shugaba(2014) found reduced the lumen diameter of the uterus and changed its normal histological from star shaped to an oval



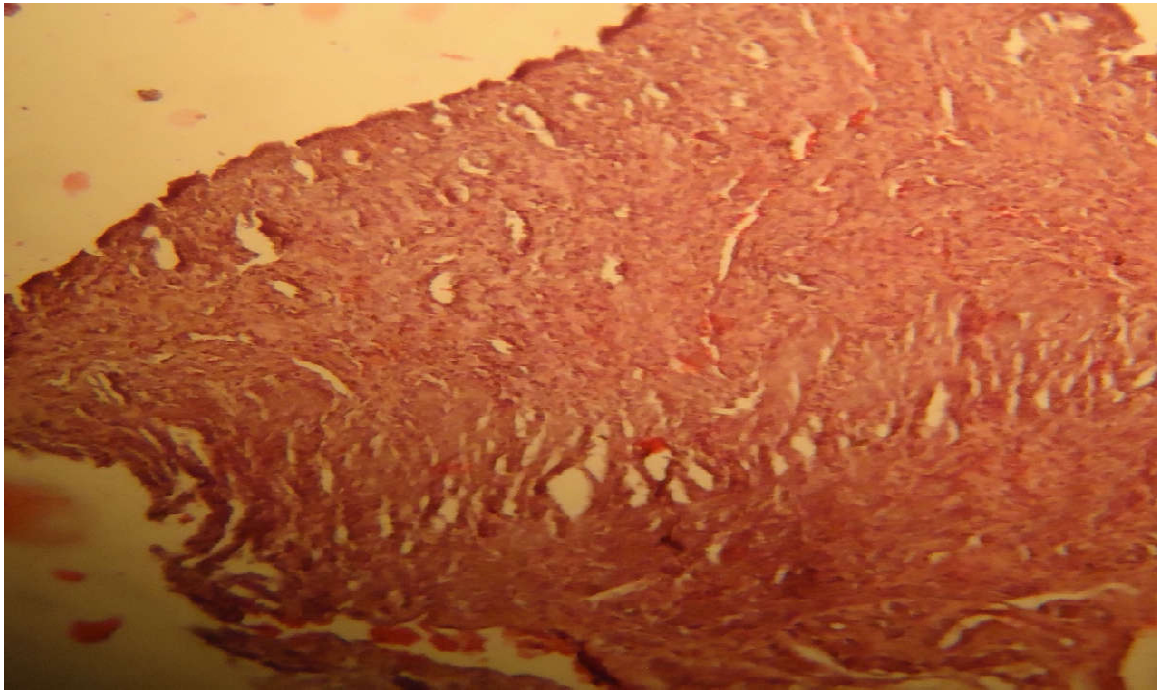


Figure 6: Section of uterus of control female rabbit shows normal tissue .H&E stain 400X

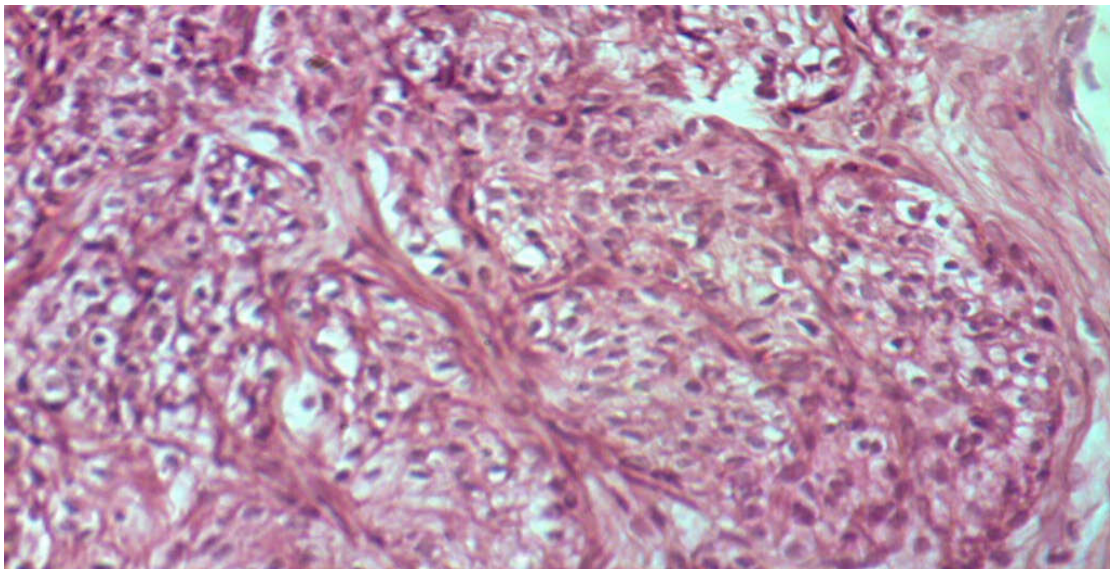


Figure7: section of uterus show clear hyperplasia of epithelial cell in uterine gland of female rabbits administrated( 500 mg/kg of B.w) from *Lawsonia inermis* .H&E(40).

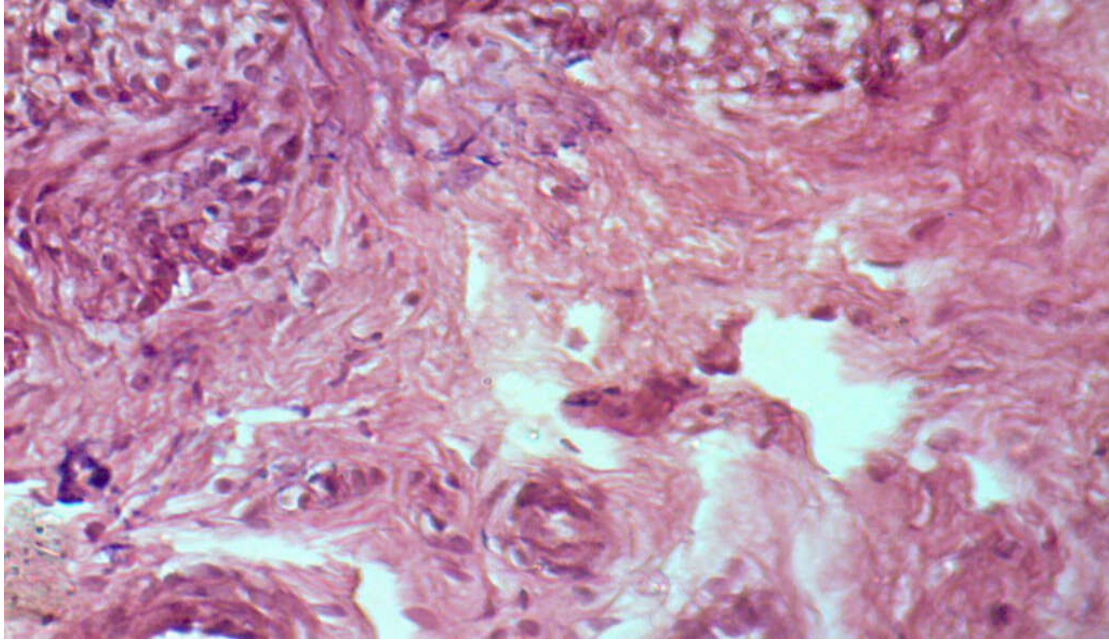


Figure 8: section of uterus of female rabbits daily treated with(1000 mg/kg B.W for 30 days shows atrophic ,fibrosis and decrease in the number of uterin gland.

## تأثير مستخلص نبات الحناء على بعض الاختبارات الدموية والكيمياء حيوية والتغيرات النسيجية لمبيض ورحم إناث الارانب

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### الخلاصة

اجريت هذه الدراسة لمعرفة تأثير مستخلص نبات الحناء على بعض الاختبارات الدموية والكيمياء حيوية والتغيرات النسيجية لمبيض ورحم إناث الارانب. أستخدم في هذه التجربة (١٨) انثى ارنب قسمت وبشكل متساوي الى ثلاثة مجاميع (٦ حيوانات في كل مجموعة). المجموعة الأولى اعطيت المحلول الفسلجي عن طريق الفم (سيطرة) حيث تم تجريعها (٣ مل من المحلول الفسلجي) والمجموعة الثانية اعطيت (٥٠٠ ملغم/ كغم من وزن الجسم) من مستخلص نبات الحناء عن طريق الفم ولمدة ٣٠ يوم أما المجموعة الثالثة اعطيت (١٠٠٠ ملغم/ كغم من وزن الجسم) من المستخلص المائي لنبات الحناء ولفترة ٣٠ يوم.

اظهرت النتائج انخفاضاً معنوياً ( $p \leq 0.5$ ) في عدد كريات الدم الحمراء والبيضاء وانخفاض في نسبة الهيموغلوبين وتركيز لكوليسترول وتركيز التراي كليسيريد وكانت النتائج اكثر وضوحاً في جرعه

١٠٠٠ ملغم/كغم من وزن الجسم مقارنه مع مجموعته السيطرة.

وجد ان نبات الحناء احدث تغيرات نسيجه مرضيه التي تضمنت فرط التنسج ونخر حاد في نسيج المبيض وحدوث تليف وضمور لنسيج الرحم لإنات الارانب في جرعه ١٠٠٠ ملغم/كغم من وزن الجسم الذي كان اكثر وضوحا من جرعه ٥٠٠ ملغم/كغم مقارنه مع مجموعته السيطرة.

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