



Research Article:

## Effect of Rosemary Extract on Some Internal Organs of Young Male Albino Rats: A Histological Study

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### Abstract

**Background:** "*Rosmarinus officinalis*" (rosemary) is a natural herb that is used by humans for the treatment of many conditions. The extract of this plant has been used as an antioxidant to improve memory, stimulate hair growth, treat asthma, treat gastric ulcers, and even, some time ago, prevent and treat cancer. **Aim:** This work was performed to study the effect of different doses of rosemary extract on some internal organs of young albino rats whose mothers were treated with rosemary aqueous extract throughout the lactation period (21 days). **Methods:** The animals were classified into three groups: the first was regarded as the control, in which mothers received no treatment, while mothers in the second and third groups were treated orally with rosemary aqueous extract (prepared by using leaves of this plant) at doses of 200 mg/kg and 1000 mg/kg respectively. At the end of the study, the young male rats from different groups were sacrificed, and their livers, kidneys, and testes were prepared for histological examination. **Results:** The physical and histological assessment of the offspring showed that treatment of lactating mothers with a high dose of rosemary extract (1000 mg/kg) affected the health of their young infants as they failed to gain weight and revealed some atrophic and degenerative changes in their liver and kidney, while the germinal epithelium of the testis is restricted to the spermatogonia and primary spermatocyte with a significant reduction in Leydig cells number compared to other groups. **Conclusion:** The research concluded that a high dose of rosemary extract, rather than a modest dose, can affect the health of young infants whose mothers were treated with this extract during lactation, so this plant has teratogenicity and should be avoided by humans during pregnancy and lactation.

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## 1. Introduction

Some natural herbs have pharmacological and biological activities, many people attempt to use herbs as a natural antioxidant to treat different medical condition (1). Recently, studies focused on herbs in the discovery of drugs because of their fewer side effects and limited complications (2) "*Rosmarinus officinalis*" (rosemary) is a type of herbs that is widely used by human. Aqueous extract of rosemary have been used as a natural antioxidant to improve memory, to

stimulate hair growth, to relieve spasmodic attack, to treat asthma, gastric ulcer and even some time for cancer prevention, treatment or both (3). The chemical components of rosemary extracts such as essential oil, carnosic, rosmarinic acid and ursolic acid as well as the carnosol have been reported responsible for the antioxidant properties of this plant (4). Liu *et al* (2016) showed that rosmarinic acid had protective effects on cardiac muscle of hypertensive rats (5). Another researcher concluded that rosemary aqueous extract administration ameliorated cardiac damage induced experimentally in rats (6). Aqueous extracts from rosemary leaves improve the quality of cow and sheep milk when added to their food (8,9). Tousson *et al* (2019) found that rosemary extracts had ameliorative effect on Etoposide induced hepatotoxicity (7). However, Nusier *et al* (2007) observed that density and motility of sperm were reduced in the testes of rosemary-treated male rats (10). On the other hand, Hamzah *et al* (2021) reported that small doses of rosemary extract had no effect on serum testosterone in hyperthyroid animals (11). Usually small doses have been

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documented to be safe even if it is taken for long time. However large amounts of rosemary extracts may cause vomiting, pulmonary edema and may be associated with congenital anomalies and abortion if it has been taken by pregnant woman. Previous research showed that high doses.

of rosemary extract given to female rats during gestation were associated with delayed fetal bone development (12). Hence, the aim of this study was to investigate the effect of rosemary aqueous extract on the health of young albino rats whose mothers were treated with different doses of this herb during lactating periods.

## 2. Material and Methods

### 2.1. Preparation of rosemary extract

*Rosemary officinalis* plant was purchased from a commercial market in Iraq, the dried leaves were grounded by electrical mixer into powder. Then 100 gm of the powder were put in 3mL of hot water. The mixture was boiled for about one hrs., after cooling the solution was mixed by electrical mixer for 10 min. thereafter the solution was filtered and evaporated at 40 °C to produce dried deposits (crude extract) which can be stored in the refrigerator and used on need (13).

### 2.2. Experimental protocol

Nine adult female rats of white albino strain at 3 months old were obtained from Veterinary College of Mosul university. The animals were placed in polystyrene cages at 20 °C temperature and fed with free laboratory food and water. Females were allowed to mate with adult male. Then pregnant rats were kept in cage till delivery after that every 3 mothers with their offspring were placed in separating cage. The animals in the first cage was regarded as the control group, the lactating mothers were given distilled water while the mothers in 2<sup>nd</sup> and 3<sup>rd</sup> cages were treated with rosemary extract at dosage of 200 mg /kg and 1000 mg/ kg/ every other day orally, respectively (14). All mothers had been allowed to free access of water and food. The treatment was continuing for about 21 days till the weaning time, then (young males number-6) from each cages were weighted and killed under anesthesia. The method of this study was built on the Ethical Values of ethical committee of Medical College/Ninevah university, the ethical license approval number 104 on February 12, 2022.

### 2.3. Histopathological examination

The animals were dissected, removed the liver, kidney and testis and fixed in 10 % formalin for 24 hours. The tissues were treated with ascending grades of alcohol, cleared with xylene and embedded in paraffin wax, followed by sectioning via microtome into 5µm thickness sections and finally staining with hematoxylin and eosin (H&E) (15). Then we used the light microscope to evaluate all prepared sections.

### 2.4. Quantitative examination

Color USB 2.0 digital image camera Omax (A3590U) provided with image processing software (TuopView 12.5) were used for micro-morphometric analysis. Five randomly chosen sections from each organ were used to estimate the following parameters:

1-In liver sections, the morphometric measurement include :  
The number of kupffer cell/46000µm<sup>2</sup>/field in liver sinusoid

2-In kidney, the morphometric measurement include: a) urinary space diameter; b) Glomerular diameter

3-In testis, the morphometric measurement include The number of Leydig cells /interstitial space.

## 2.5. Statistical analysis

The data were expressed as mean± standard error (SE). The statistical analysis was performed by Graph pad prism program (version 9.2.0). Values of  $p \leq 0.05$  was regarded significant statistically. ANOVA (one way analysis) followed by Student –new man-Keuls multiple comparison test was used in this study.

## 3. Results and observations

1- **Body weight:** The weights of young rats whose mothers were treated by the high dose of rosemary extract (1000 mg/kg) showed a significantly ( $p \leq 0.05$ ) reduction compared to that of control while the body weights of those in group II showed non-significant ( $p > 0.05$ ) differences from those of control group as shown in **Table 1**.

2- **Histological findings**

**A-Liver:** liver sections of control group showed normal liver structure which consist of many lobule. Each lobule has normal central venue, hepatocytes arranged as plates which run between the central venule and the periphery of the lobule. Portal traid are seen in the peripheral of the liver lobule (**Figure 1. a and b**). The liver sections from group 2 showed histological structure similar to that of control group (**Figure 1. c and d**). However, the sections of liver from group 3 showing disturbance in liver architecture characterized by dilatation and congestion of central vein and sinusoids with foci of hemorrhage, vacuolar degeneration of many hepatocytes, infiltration of mononuclear inflammatory cells within the hepatocytes, sinusoids and in portal tract and hyperplasia of kupffer cells in the sinasoid (**Figure 1. e, f and g**).

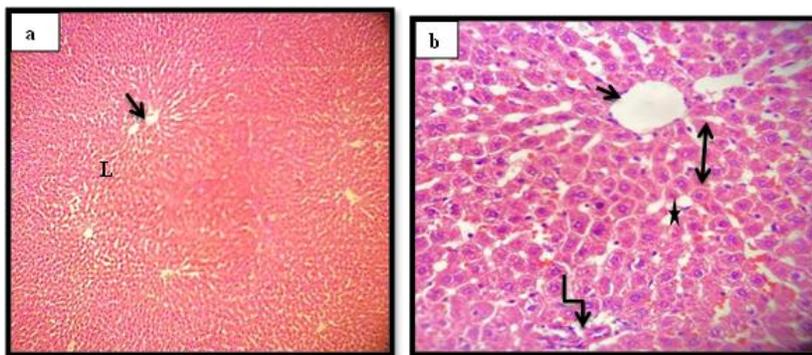
**B- Kidney:** the kidney sections from control group shows normal appearance. It consists of cortex and medulla. In the cortex, the renal corpuscle consists of tuft of blood capillaries (glomerulus) surrounded by Bowman's capsule which has an outer layer of simple squamous epithelium and an inner visceral one separated by a rental space. In addition to the renal corpuscles, the cortex also contains proximal convoluted tubule which is lined by cuboidal epithelium and distal convoluted tubule that lined by cuboidal epithelium lighter than those of proximal

tubule (**Figure.2 a and b**). Light microscopic study of sections from group 2 show no deviation in histological findings from those seen in control group (**Figure.2 c and d**). On the other hand, the sections of kidney taken from group 3 show glomerular atrophy with widening of renal space. Some proximal tubule show degeneration of their epithelium (**Figure.2 e and f**).

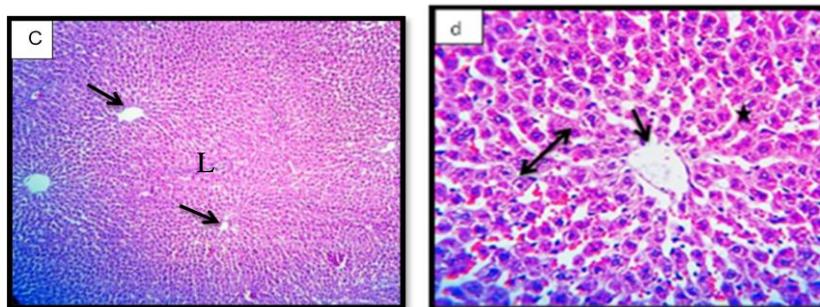
**Table 1** . Changes in the body weight in different groups of young rats

Variables	Groups and Body weight (mean (gm)± SE)		
	Group 1 N=6	Group II N=6	Group III N=6
1 day old	3.95±1.6	3.98±2.4	4.12±0.84
21-day old	35.7±5.23	18.2±5.13a	39.7±3.31
p-value	p<0.001	p<0.01	p<0.001

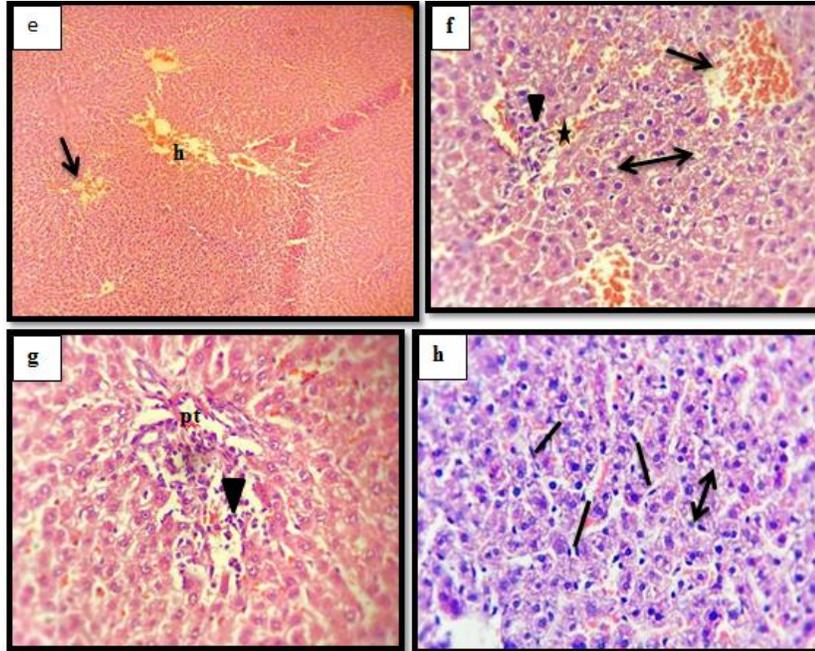
SE: stander error of mean , a-indicates significant difference from other groups at  $p \leq 0.05$



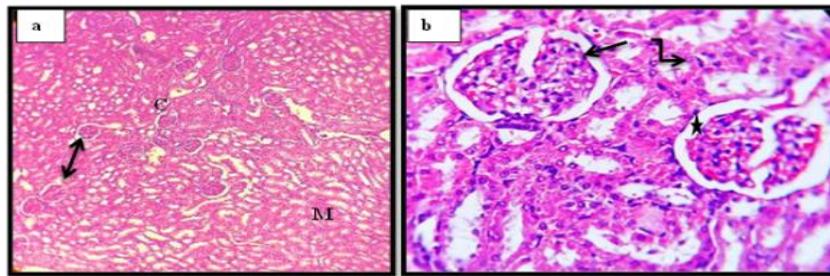
**Figure 1.** Photomicrographs of liver from control group showing normal liver structure consist of lobule (L). each lobule has central venule ( arrows ) , hepatocytes plate ( bihead arrow) separated by sinusoid ( star) and radiat from cental to peripheral. Portal traid ( curved arrow ) are detected H&E a ,100X. b,400X.



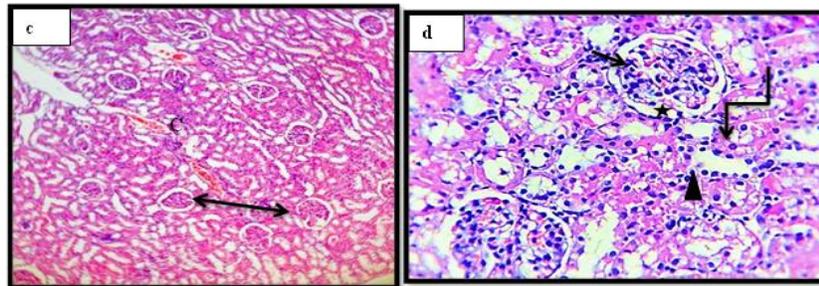
**Figure 1.** Photomicrographs of liver from group (2) showing normal liver lobule (L), each lobule has central venule ( arrows ) , hepatocytes plate ( bihead arrow) separated by sinusoid ( star) and radiat from cental venule to peripheral. H&E c ,100X. d,400X.



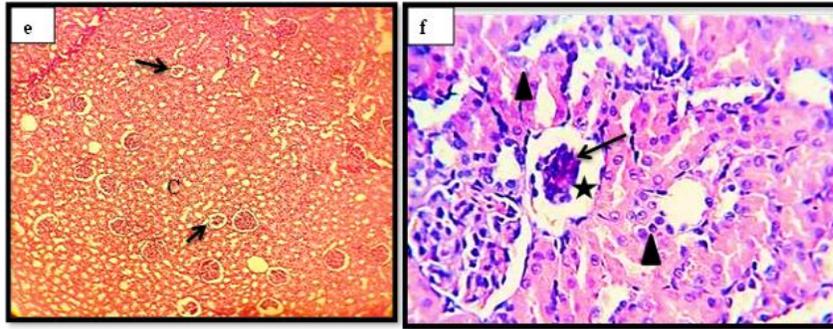
**Figure 1.** Photomicrographs of liver from group (3) showing dilatation and congestion of central venule (arrows) and sinusoid (star), foci of hemorrhage (h) vacuolar degeneration of some hepatocytes (bihead arrow) , inflammatory cells infiltration (head arrows) of hepatocyte , sinusoid and portal tract (pt) hyperplasia of kupffer cells ( (black line). H&E e ,100X. f,g,h,400X.



**Figure 2.** Photomicrographs of kidney from control group showing renal cortex (C) and medulla (M) , cortex contains many renal corpuscle (bihead arrow) which consist from glomerulus ( arrow) surrounded by two layers of Bowman's capsule separated by renal space (star);proximal tubule ( curved arrow) and distal tubule ( head arrow ) present in the cortex . H& E. a, 100X. b, 400X



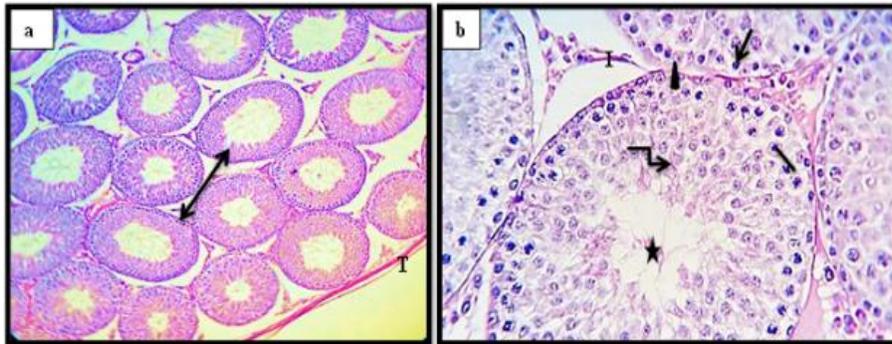
**Figure 2.** Photomicrographs of kidney from group II showing renal cortex (C) contains many renal corpuscle (bihead arrow) which consist from glomerulus (arrow) separated from Bowman's capsule by renal space (star).proximal tubule (curved arrow) and collecting tubule (head arrow) present in the cortex . H& E. c, 100X. d, 400X



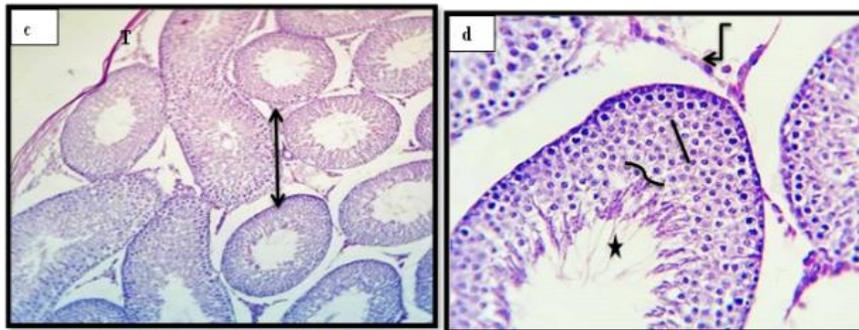
**Figure 2.** Photomicrographs of group III rat's kidney show renal cortex (C) glomerular atrophy (arrows) with widening of renal space (star). Some proximal tubule show degeneration of their epithelium (head arrows) . H& E. e, 100X. f, 400X

**C-Testis:** The sections of control group microscopically show normal features of testis , in which the testis is made up of rounded -oval seminiferous tubule separated by testicular interstitium containing clump of Leydig cells. Each tubule is lined by spermatogenic and sertoli cells. The spermatogenic cells consist of small cells resting on the basement membrane of the tubule (spermatogonia) followed by large rounded cells (primary spermatocytes ) while the

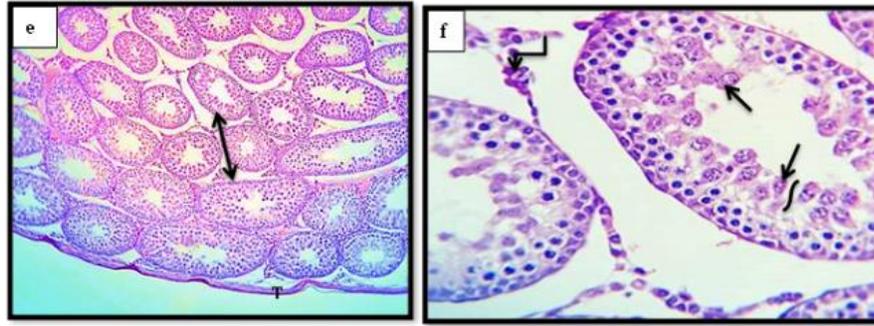
spermatid appear as small cells near the lumen and the few sperm in the lumen (Figure.3 a and b). While histological finding of the testis from group 2 (**Figure 3 c and d** ) are similar to those of control group, the sections from group 3 show arrest of spermatogenesis at the level of primary spermatocytes and the interstitium contains few Leydig cells which some of them has small pyknotic nucleus (**Figure.3 e and f**).



**Figure 3.** Photomicrographs of rat 's testis group (1) showing tunica albugenia (T) , rounded -oval seminiferous tubules separated by interstitium ( I) containing Leydig cells ( curved line) , the lining epithelium of seminiferous tubule consist of spermatogenic cell ( arrow) , sertoli cell ( head arrow) , primary spermatocyte( black line), spermatid ( curved arrow) and sperm (star) fill the lumen. H& E a,100X. b,400X.



**Figure 3.** Photomicrographs of rat 's testis group (2) showing tunica albugenia (T) , rounded -oval seminiferous tubules (separated), the lining epithelium of seminiferous tubule consist of spermatogenic cell, spermatocyte( black line), spermatid (curved line) and sperm (star) in the lumen . Leydig cells (curved arrow) present between seminiferous tube . H& E c,100X. d,400X.



**Figure 3.** Photomicrographs of rat 's testis group (3) showing tunica albugenia (T) , seminiferous tubules (bihead arrow), germinal epithelium consists of spermatogonia resting on the basement membrane (black line) and primary spermatocyte (arrows) with intracellular space (curved line), no sperm in the lumen. Leydig cells with pyknotic nucleus (curved arrow). H&E e,100X. f,400X.

**3- Quantitative (morphometrical) findings:**

- A- The morphometrical analysis of the liver sections revealed a significant ( $p \leq 0.05$ ) elevation of kupffer cells number in young rats whose mothers treated with high dose of rosemary extract ( 1000 mg /kg) as compared to other groups **Table 2**.
- B- The morphometric study on the diameter of renal space in renal corpuscle showed a significant

increase at  $p < 0.05$  in group 3 compared to group 1 and 2. However, measurement of the glomerular diameter reveal a significant decrease in group 3 compare to other groups **Table 3**.

- C- The morphometric analysis of Leydic cells count in the testis of different groups showing a significant reduction of cells number in group 3 as compared to other groups **Table 4**.

**Table 2.** Effect of rosemary extract on the number of kupffer cells of liver in different groups

Number of kupffer cell (mean ±SE)	Groups		
	Group 1 N=6	Group II N=6	Group III N=6
	16.57±1.04	18.14±0.60	26.57±1.61 a

SE: stander error of mean ,a: significant difference from other groups at  $p < 0.05$  .

**Table 3.** Effect of rosemary extract on the renal space diameter and glomerular diameter in different groups.

Parameters (mean±SD)	Groups		
	Group 1 N=6	Group II N=6	Group III N=6
Renal space diameter	5.22±0.23a	7.14±0.11a	17.70±2.61 b
Glomerular diameter	104.37±5.63a	96.80±0.05a	67.18±5.37b

The different letters indicate a significant difference at  $p \leq 0.05$ . The similar letters mean non- significant difference at  $p > 0.05$

**Table 4.** Estimation of Leydig cells number in different groups. Mean

Number of Leydig cells (mean±SE)	Groups		
	Group 1 N=6	Group II N=6	Group III N=6
Leydig cells count/ 46000µm/ field	9.72±0.43a	8.14±0.51a	4.80±2.61 b

The different letters indicate a significant difference at  $p \leq 0.05$ . The similar letters mean non- significant difference at  $p > 0.05$

## 4. Discussion

Pregnancy and lactation are critical periods of fetal and infant development, the maternal health and diet may have direct influence on the growth and health of their babies (16). Most aromatic and herbal medicines, which have been used by many persons during lactation either for improvement of mother's health or stimulation of milk production, are built on individuals experiment and not on scientific research of safety and effectiveness of these herbs (17). Rosemary is a medicinal herb that has been used for many years ago as food approval as well as in medical field for treatment of gastric distress, headache, depression and many other condition (18).

The current study showed a significant reduction in the weight of the young animals whose mothers had been treated with high dose of rosemary. While those young rats whose mothers were treated with low dose showed normal growth with their weights non-significantly differ from that of control group. Because breast milk is considered the main source of infant nutrition during lactation, so its composition may affect the health and growth of the infant (19). However, the milk composition is affected by the maternal intake and because the fact that rosemary extract contains many chemical materials which may be associated with changes of some metabolic hormone if it is taken in large quantity and as a consequence it affects the infant health (20,21).

The histopathological finding in the present study indicates that treating lactating mothers with low dose of rosemary extract induced no changes in the organs of young offspring while larger dose induced many pathological changes which include vacuolar degeneration, congestion of blood vessel, disturbances of normal architecture and even atrophy of some structures in kidney and hyperplasia of other in the liver while the testis showed arrest of spermatogenic cells on primary spermatocyte stage. Motaghi *et al* (2021) found that treatment of adult rats with rosemary oil for 30 days caused elevation of liver enzyme and deterioration of renal function associated with atrophic changes of both renal and liver tissues.

The action of rosemary extract is controversial. Mohamed *et al* (2022) observed that rosemary extract had hepatoprotective effect against fibrosis and degeneration induced experimentally in female rats (23). Saied *et al* (2023) showed that rosemary oils improved spermatogenesis and elevated the level of testosterone in male rats who had testicular dysfunction induced by Etoposide (24). On the other hand, Nusier *et al* (2007) denied the effectiveness of rosemary on improvement of male fertility (10). While other said that rosemary had no effect on testosterone level and male sexuality of aged rats (25). However, the doses and chemical constituents of rosemary extract may be responsible for this controversial effect. Cineole is regarded as the most abundant component of rosemary extract (26). Xu *et al* (2014) concluded that cineole had toxicity when used for long time and caused destruction of mitochondria, endoplasmic reticulum and other organelles of renal and liver cells (27). Another study observed that rosemary extract particularly cineole induced fetal toxicity and histological changes in the liver, uterus and lungs of female rats (28). Accordingly, the histopathological changes detected in the current study maybe connected to the composition of rosemary extract particularly cineole.

## 5. Conclusion

The data obtained from this work showed that oral administration of rosemary extract to the lactating mothers was safe when given in small dose however the large dose could affect the infant health through its effects on the function of some vital organs. Therefore, the usage of this herbs should be restricted during pregnancy and lactation period in human.

## 6. Acknowledgments

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## 7. Conflict Of Interest

There is no conflict of interest

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## تأثير مستخلص إكليل الجبل على بعض الأعضاء الداخلية لصغار الذكور الجرذان البيضاء: دراسة تيسيرية

الخلاصة:

**المقدمة:** (إكليل الجبل) هومن الأعشاب الطبيعية التي يستخدمها الإنسان لعلاج العديد من الحالات ، وقد استخدم مستخلص هذا النبات كمصدر لمضادات الأكسدة لتحسين الذاكرة ، وتحفيز نمو الشعر وتخفيف النوبات التشنجية ، وعلاج الربو وقرحة المعدة وللوقاية من السرطان وعلاجه . **الهدف من الدراسة:** تم إجراء هذا العمل لدراسة تأثير الجرعات المختلفة من مستخلص إكليل الجبل على بعض الأعضاء الداخلية للفران الصغيرة البيضاء التي عولجت أمهاتها بمستخلص إكليل الجبل طوال فترة الرضاعة والتي بلغت حوالي 21 يوماً. **الطريقة:** في هذه التجربة تم تصنيف الحيوانات إلى ثلاث مجموعات ، المجموعة الأولى اعتبرت مجموعة السيطرة حيث لم تتلق الأمهات أي علاج بينما عولجت الأمهات في المجموعتين الثانية والثالثة عن طريق الفم بمستخلص إكليل الجبل بجرعة 200 ملغم/كغم و 1000 ملغم/كغم على التوالي. **النتائج:** أظهرت نتيجة هذه الدراسة أن علاج الأمهات المرضعات بجرعة عالية من مستخلص إكليل الجبل يؤثر على صحة أطفالهن الصغار حيث فشلوا في زيادة الوزن وكشفت الدراسة عن بعض التغيرات الضمورية والتكسية في الكبد والكلية بينما انحسرت الظهارة الجرثومية للخصية إلى الخلايا المنوية والخلايا المنوية الأولية مع انخفاض كبير في عدد خلايا Leydig مقارنة بالمجموعات الأخرى. **الخلاصة:** خلص البحث إلى أن الجرعة العالية وليس الجرعة القليلة من مستخلص إكليل الجبل يمكن أن تؤثر على صحة الرضع الصغار الذين عولجت أمهاتهم بهذا المستخلص أثناء الرضاعة.

الكلمات المفتاحية: إكليل الجبل، مستخلص، جرذان ألبينو، دراسة التيسير المرضي