

## Effect of *Salvia Officinalis* on the Histological Parameters and physiological creteria of Male Reproductive System in Mice

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### Abstract

The present study aims to reveal the effect of *Salvia officinalis* in the physiological and histological state of male mice reproductive system. Fifteen adult males Swiss Albino mice at the age of two months were divided into three groups; the control group and the experimental group, which is subdivided into two groups. The first treated group received 0.03g/day extract of *Salvia officinalis*, the second was treated with 0.01g/day extract of *Salvia officinalis* for 30 days. Then, the animals were weighted and dissected to remove the testis for histological study. Also, the liver, kidney, heart, epididymis, prostate and spleen were removed from the animals and weighted. The study revealed an increase in body weight of animals treated with *Salvia officinalis*. There was a significant gradual increase in body weight (28.56, 31.88)g which was of direct proportion with the increase of the given dosages of the Salvia as compared with the control group 27.65g. The testis and epididymis showed a clear increase in weight (0.14, 0.736, 0.298, 0.526)g which was statistically significant ( $P < 0.05$ ) and this increase was in direct proportion with the increase in the given dosages of Salvia when compared with the control group (0.116, 0.262)g. There was a significant increase ( $p < 0.05$ ) in the weight of the liver, spleen, kidney and prostate (1.68, 1.89, 0.256, 0.306, 0.454, 0.552, 0.206, 0.276)g as compared with the control group (1.44, 0.212, 0.436 and 0.176)g, respectively. Histological examination showed an increase in the number of layers in germinative epithelia of seminiferous tubules. On the other hand, results showed an increase in the number of sperms within the lumen in addition to an decrease in the diameter of tubules as compared with the control group and the activation of tubules epithelium increases with the increase of dose. These results highlight the role of *Salvia officinalis* in activating spermatogonia and the production of sperms.

تأثير نبات المريمية *Salvia officinalis* على بعض المعايير الفسلجية والنسجية للجهاز

التناسلي الذكري في الفئران

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### الخلاصة

تهدف الدراسة إلى الكشف عن أهمية نبات المريمية *Salvia officinalis* لبعض الحالات الفسيولوجية والنسجية للجهاز التناسلي الذكري في الفئران. شملت الدراسة 15 فأراً من نوع Albano mice بعمر شهرين. وقد قسمت الحيوانات إلى ثلاثة مجاميع: مجموعة السيطرة ومجاميع الاختبار التي قسمت إلى مجموعتين: الأولى استلمت 1% من مسحوق أوراق المريمية. إما المجموعة الثانية استلمت 3% من مسحوق أوراق المريمية لمدة 30 يوماً. بعدها الحيوانات وزنت وشرحت لإزالة كل من الخصية والبربخ لغرض الدراسات النسجية. كذلك استأصلت كل من الكبد والقلب والطحال والبروستات ثم وزنت. أظهرت نتائج الدراسة الحالية

وجود زيادة معنوية في وزن الجسم للحيوانات المعاملة بالمريمية وان هذه الزيادة كانت تدريجية في وزن الجسم تتناسب طرديا مع الجرعة المعطاة من النبات (28.56، 31.88) غم بالمقارنة مع مجموعة السيطرة 27.65 غم على التوالي. أما الخصية والبربخ فقد اظهروا زيادة واضحة في الوزن (0.14, 0.736, 0.298, 0.526) غم وهذه الزيادة كانت معنوية عند ( $p < 0.05$ ) وهذه الزيادة تتناسب طرديا مع الجرعة المعطاة بالمقارنة مع مجموعة السيطرة (0.116, 0.262) غم. كما أظهرت الدراسة وجود زيادة معنوية ( $p < 0.05$ ) في وزن كل من الكبد والطحال والكلية والقلب والبروستات (0.276, 0.206, 0.23, 0.18, 0.552, 0.454, 0.306, 0.256, 1.89, 1.68) غم بالمقارنة مع مجموعة السيطرة (0.176, 0.156, 0.436, 0.212, 1.44) غم على التوالي. ومن الناحية الفسلجية ظهرت زيادة معنوية في عدد طبقات الخلايا الطلائية للنبيبات المنوية. ومن ناحية أخرى ظهرت زيادة في عدد النطف ضمن التجويف، بالإضافة إلى نقصان في قطر النبيبات بالمقارنة مع مجموعة السيطرة، وان فعالية الخلايا الطلائية للنبيبات تزداد بزيادة الجرعة المعطاة. ان هذه الدراسة تعطي مؤشر واضح على دور المريمية في تنشيط عملية تكوين وإنتاج النطف.

### Introduction

Several studies have shown that there are about 900 species of *Salvia* worldwide including *Salvia officinalis*, the most common species (1, 2), which is native to the Mediterranean region and is commonly known as sage. The infusion and decoction of the leaves have been used as nerve tonic, digestive, antispasmodic and anti-inflammatory (3). *Salvia* contains chemical components such as: cineol, borneol, pinene, flavonoids, saponin, glycoside, resin, vitamin C and E, rosmarinic acid, chlorogenic acid, caffeic acid, steroids and tannin (4, 5). Recent studies have found that *Salvia* has positively physiological effects on heart (6, 7), liver and kidney function (8, 9). Research also has shown that *Salvia* has anxiolytic (10), anti-inflammatory (11, 12), antifungal (13), antimicrobial (14,15) antioxidant (16) and antihyperglycemic properties (17,18). Several investigations have shown that many of these plants have antioxidant activities that could be therapeutically beneficial and it has been mentioned that the antioxidant potential of plants might be due to their phenolic components. It is well known that oxidative stresses induced by oxygen-free radicals and resultant tissue damage are the hallmarks of several chronic disorders and cell death (19). The therapeutic potentials of the medicinal plants are that they represent natural antioxidants in reducing such free radical induced tissue damage (20), and in the maintenance of health and protection from some age-related degenerative disorders. Sage is also used internally in the treatment of excessive lactation, night sweats, excessive salivation (as in Parkinsons disease), profuse perspiration (as in tuberculosis), anxiety, depression, female sterility and menopausal problems. The essential oil from the plant is used in small doses to remove heavy collections of mucous from the respiratory organs and mixed in embrocations for treating rheumatism. The leaves make excellent tooth cleaners, have antiseptic properties and can heal diseased gums (21). Leaves' extract appeared with antimicrobial effect against bacteria and fungi including dermatophyte (22). The aim of study effect of important and role of *Salvia officinalis* in the physiological and histological state of male mice reproductive system.

### Materials and Methods

- **Animals and experimental design:** Fifteen healthy 8-week-old male Swiss Albino mice weighting approximately 22-28g were purchased from the National Center for Researcher. The animals were housed in stainless steel cages individually in a controlled temperature room ( $25 \pm 2^\circ\text{C}$ ) and a 12-h light/ 12-h dark cycle. The animals were provided with a standard mouse diet ad libitum.

- **Experimental design:** Fifteen male mice were randomly divided into three groups:  
Group I : Non exposed animals (control).  
Group II: 1% g of Salvia/ (100g diet) treated for 30 days.  
Group III : 3% g of Salvia/ (100g diet) treated for 30 days.
- **Histological Section and parameters:** The animals were weighted and dissected to get the testis and other organs and the weighted testes were kept in formalin (10%) for fixation for 24 hours, then passed through a gradual concentration of alcohol, embedded in paraffin and cut by microtome at 5µm in thickness. Serial sections per testis were mounted on slides, deparaffinized, rehydrated and stained by hematoxylin-eosin stain. Sections were examined by a light microscope. Lumen diameter and the thickness of germinal epithelia of seminiferous tubules were assessed by using calibrated micro meter (Ocular micrometer, Stage micrometer).
- **Statistical analysis:** Statistical analysis was performed by on way analysis of variance (ANOVA) test. The difference among groups was considered significant when ( $p < 0.05$ ).

### Results

Different photochemicals have various protective and therapeutic effects which represent an essential oil to prevent diseases and maintain a state of well being. The present study has shown that *Salvia officinalis* significantly increased the body weight gain (28.56, 31.88)g which was a direct proportion with the increase of the given dosage as compared with the control group 27.65g. The liver is the main organ responsible for metabolism and detoxification. The present study has recorded a significant increase in the weight of the liver (1.68, 1.89)g as compared with the control group. The results of this study also revealed that there was a significant increase in the weight of heart, kidney, spleen, testis, Epididymis and prostate (0.18, 0.23, 0.454, 0.552, 0.256, 0.306, 0.14, 0.73, 0.298, 0.526, 0.206, 0.276)g, respectively as compared with the control group (0.156, 0.436, 0.212, 0.116, 0.262, 0.176)g, respectively. The histological study showed that *Salvia officinalis* significantly increased in thickness of epithelium (34.39, 37.12) as compared with the control group 19.52. The result of this study also revealed significantly decreased in diameter of lumen (21.96, 19.72) as compared with control group 38.08.

**Table (1) Effect of *Salvia officinalis* on body weight, heart, liver and spleen**

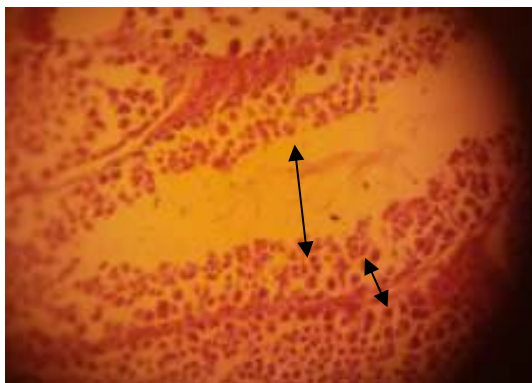
Spleen (g)		Liver (g)		Heart (g)		Body weight (g)		
SD±	mean	SD±	mean	SD±	Mean	SD±	Mean	
0.008	0.212	0.062	1.44	0.015	0.156	1.18	27.65	Control
0.025	0.256	0.205	1.68	0.016	0.18	2.20	28.56	1%
0.015	0.306	0.231	1.89	0.021	0.23	1.86	31.88	3%
P<0.05		P<0.05		P<0.05		P<0.05		P

**Table (2) Effect of *Salvia officinalis* on kidney, testis, Epididymis and prostate**

Prostate (g)		Epididymis (g)		Testis (g)		Kidney (g)		
SD±	mean	SD±	mean	SD±	mean	SD±	Mean	
0.017	0.176	0.046	0.262	0.027	0.116	0.027	0.436	Control
0.015	0.206	0.045	0.298	0.037	0.14	0.038	0.454	1%
0.121	0.276	0.44	0.526	0.144	0.73	0.038	0.552	3%
P<0.05		P<0.05		P<0.05		P<0.05		P

**Table (3) The histological parameters of Epithelium thickness and diameter of lumen of semineferous tubules**

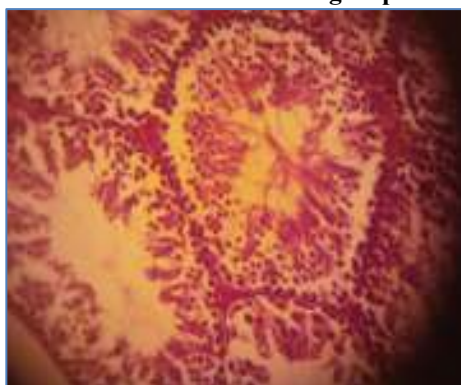
Control				Treated group 1				Treated group 2			
Thickness of epithelium		Diameter of lumen		Thickness of epithelium		Diameter of lumen		Thickness of epithelium		Diameter of lumen	
SD±	mean	SD±	Mean	SD±	mean	SD±	mean	SD±	Mean	SD±	Mean
3.11	19.52	1.829	38.08	2.73	34.36	3.759	21.96	4.22	37.12	1.890	19.72
P<0.05		P<0.05		P<0.05		P<0.05		P<0.05		P<0.05	



**Fig. (1)** Showed the thickness of epithelium and diameter of lumen of seminiferous tubules in the control group



**Fig. (2)** Showed the thickness of epithelium and diameter of lumen of seminiferous tubules in the treated group with 1% of plant extract



**Fig. (3)** Showed the thickness of epithelium and diameter of lumen of seminiferous tubules in the treated group with 3%g of plant extract

### Discussion

The study showed that administration of *Salvia* extracts has resulted in increased body weight of the organs from mice including the testis. The results have highlighted the effects of *Salvia* on the body especially male reproductive system. Fig 1, Fig 2 showed a significant increase of weights as compared with the control group. These results might be due to the effect of *Salvia* components, in particular vitamins C and E, flavonoids, phenolic compounds and antioxidants. These components leads to regulation of signal transduction pathway of cell growth and proliferation, induction of apoptosis, modulation of enzyme activity related to detoxification, oxidation and reduction, stimulation of the immune system and DNA repair and regulation of hormone metabolism (23). The histological aspects in this study revealed an increase in the thickness of epithelium and a decrease of lumen of seminiferous tubules in the treated group with the increase in dose as compared with the control group. Fig 3 shows the distribution of histological parameters among experimental groups. These results may be due to the effect of plant extract on testosterone which affects the formation of type A spermatogonia and conversion of spermatocyte into a secondary spermatocyte and on interstitial cell stimulating hormone (ICSH) or spermatogenic cell stimulating hormone (SSH) responsible for the final steps of maturation of spermatids. These results conform with those of Kulood et al., (24). The results in this study pertaining to histological parameters may be due to the properties of *salvia* plant which contains steroidal saponins. Also, ethanolic extract with essential oil of *salvia* was shown to have certain effect on fertility and that is why many parameters under study gave positive result for *salvia* effects (25). Other studies have shown that antioxidants within *salvia* extract can stimulate the normal function of leydig cells (26,27). Moreover, the presence of vitamins C and E also has a beneficial effect in the treatment of male infertility (28,29). It was concluded from this study that the use of *Salvia officinalis* might be increase fertility in male mice.

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