

The effect of Ethyl acetate and Ethyl alcohol extract of Pepper Capsicum spp. in the fertility of Albino rats females

تأثير مستخلصي خلات الأثيل والكحول الأثيل لثمار الفلفل الأحمر في خصوبة إناث الجرذان البيضاء

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Abstract

This study was conducted to investigate the effect of biochemical activity of Ethyl acetate and Ethyl alcohol extracts effect of Pepper Capsicum spp. in the fertility of Albino rats females in a number of (75) rats, females were divided into three groups and given various doses (50 and 100) mg/kg of each extracts and were compared with a group of control in three injection periods (5, 10, 15 days).

- The results of females fertility test showed that no sperms in the vaginal space swab before a time period and delay in pregnancy period through daily following up for (30) days and a significant decrease in fetuses number of female rats treated with both extracts for injection period of (12) days of Ethyl acetate and Ethyl alcohol extracts in comparison with a control group.
- Histological study has showed significant decreased ($p < 0.05$) in the diameters of ovaries and a the number of primary and secondary ovarian follicles and the Graafian ovarian follicles numbers and diameters of female rats treated with both extracts, Ethyl acetate and Ethyl alcohol in comparison with a control group.
- A significant increased ($p < 0.05$) in the total of the body weight regarding rats females treated with only Ethyl acetate extract in comparison with a control group, while there isn't any significant changed in the total of the body weight of the rats females treated with Ethyl alcohol extract.
- A significant decreased ($p < 0.05$) in the body weight of ovaries and a highly significant ($p < 0.05$) in the weight of uterus of females treated with both extracts, Ethyl acetate and Ethyl alcohol in comparison with a control group, Ethyl acetate showed a higher activity in decreasing of ovaries weights and the increase of uterus weights in comparison with Ethyl acetate extract.

الخلاصة

- أجريت هذه الدراسة لتقصي تأثير الفعالية الكيموحيوية لمستخلصي خلات الأثيل والكحول الأثيل لثمار الفلفل الأحمر في خصوبة إناث الجرذان وبواقع (75) جرذاً، قسمت على ثلاث مجاميع وأعطيت كلا التركيزين من كل مستخلص وحسب معدلاتها (50، 100) ملغم/كغم وفورنت مع مجموعة السيطرة ولثلاث مدد من الحقن (5، 10، 15) يوماً.
- أظهرت النتائج إختبار خصوبة الإناث عدم وجود النطف في المسحة المهبلية إلا بعد مرور مدة من الزمن وحدث تأخر في مدة الحمل من خلال المتابعة اليومية ولمدة (30) يوماً ووجود انخفاض واضح في أعداد الاجنة لإناث الجرذان المعاملة بكلا التركيزين ولمدة حقن أمدها (12) يوماً لمستخلصي خلات الأثيل والكحول الأثيل مقارنة مع مجموعة السيطرة.
 - أظهرت الدراسة النسيجية إنخفاض معنوي ($p < 0.05$) في أقطار المبايض وأعداد الجريبات المبيضية الأولية والثانوية وجريبات كراف واقطارها لإناث الجرذان المعاملة بكلا التركيزين لمستخلصي خلات الأثيل والكحول الأثيل مقارنة مع مجموعة السيطرة.
 - وجود زيادة معنوية ($p < 0.05$) في وزن الجسم الكلي بالنسبة لإناث الجرذان المعاملة بمستخلص خلات الأثيل فقط مقارنة مع مجموعة السيطرة بينما لم يحدث أي تغيير معنوي في وزن الجسم الكلي لإناث الجرذان المعاملة بمستخلص الكحول الأثيل.
 - وجود إنخفاض معنوي ($p < 0.05$) في أوزان المبايض وزيادة معنوية ($p < 0.05$) في أوزان الأرحام لإناث

الجرذان المعاملة بكلا التركيزين لمستخلصي خلايا الاثيل والكحول الاثيل مقارنة بمجموعة السيطرة، وكان هناك فعالية عالية لمستخلص خلايا الاثيل في انخفاض أوزان المبايض وزيادة أوزان الارحام مقارنة مع مستخلص الكحول الاثيل.

Introduction

Pepper Capsicum spp. is considered as one of widespread medical plants; it belongs to the Piperaceae (1) its active components, specially Alkaloids were used in treating of many diseases infecting of many systems in the body such as digestive, respiratory and circulatory system (2). Many researches were directed toward using of medical plants as contraceptin females due to activity they have showed and as a result to their little side effects in comparison with contraceptbills, thus, their usage as contracepthas entered the stage of active execution, the manufacturing companies started promoting them in markets as alternatives of contraceptbills.

- The study of Ethyl acetate and Ethylic alcohol extracts effects of Pepper Capsicum spp. L. in the fertility of white female rats in respects of: Total body weight, Weights of reproductive systems (ovaries and uterus), Testing of females fertility through the accounting of the first day of pregnancy, pregnancy period and the number of fetuses.
- Histological study this included accounting the numbers of Primary follicles, Secondary follicles and Graffian follicles, their diameters and ovaries diameters, also measuring of uterus layers thickness (uterus muscle and endometrium).

So that the main aim of this research is; investigating about the activity of Pepper Capsicum spp. L. in order to know this role as pregnancy preventer.

Materials and Methods

Preparing of lab. animals

The study included using of Albino rats females of in ages between (9-13 weeks), the rate of their weights was (250-300 g), have also been used after making sure of their fertility in order to make fertility test. These animals were put in animal house circumstances in a temperature between (21-30 C°) under fixed lighting system in a rate of (12 light hours) and (12 dark hours).

The animal females were vivisected after being anesthetized with Chloroform on the (7 day), regarding the groups which been injected with different concentrations of both extracts for a period of (6 days), while the females which were injected with extract concentrations for a period of (9 days), they were vivisected on the (14 day). Abdominal cavity was opened and the different organs of female reproductive system were removed, including ovaries and the two horns of uterus, they were put in formalin solvent (10%) in order to remove fatty materials adhered with them which were dried by filtering papers. Then, they were weighted by a sensitive scale type (Germany, Sartorius), after that histological and physiological phenomenon were studied according to the designed experiments of this study. Rats females weights were measured before and after the end of experiment (5,10,15 days), they were compared with control group through the usage of a normal scale, while reproductive systems (ovaries and uterus) they were removed after being killed and then weighed by a sensitive scale. The numbers of primary, secondary and Graffian ovarian follicle were accounted in a form of serial slices of all extract concentrations in both types Ethyl acetate and Ethyl alcohol according to their rats in three rats females injected with the same concentration and then compared with control group. Uterus layers thickness was measured in rat females through using the precise scale of the optic lens after being measured with precise scale of the scene in enlarging power of x10 ($x_{10} \times 10 \times 1.250 = 125$) then compared with control group.

Preparing of Pepper Capsicum spp. organic solvents:

Organic solvents (Ethyl acetate and Ethyl alcohol extracts) of Pepper Capsicum spp. were prepared in accordance to method of (3,4), where (20g) of dried Pepper Capsicum spp. were taken, materials were extracted consequently of it by Soxhlate consequent extraction apparatus with (200 ml) of each solvent starting from Ethyl acetate then Ethyl alcohol for (24hours) each separately, after that, extracted significant was concentrated by rotated evaporator in a temperature

of (40-45 C°) in order to evaluate the vital activity of solvents extract, after that (2 g) of dry extracted significant of each solvent were dissolved separately in (10ml) of filtered water to obtain a stock solution of (0.2 g/ml). this operation was repeated many times to obtain plentiful active significant , then concentrations of (50,100)mg/kg of the total body weight were prepared

The Results:

Table (1) showed a significant increase in the total body weight after being treated with Ethyl acetate of Pepper Capsicum spp. of both concentrations in comparison with control group, where it reached (23.4), the concentration (100) mg/ kg showed a significant increase in the total body weight after being treated with Ethyl acetate of Pepper Capsicum spp. of both concentrations, the results has showed that Ethyl acetate extract has affected on only the total body weight in comparison with control group, where the data of statistical analysis results appeared on the those existed significant differences.

Table (1): The effect of interference of various concentrations of Ethyl acetate and Ethylic alcohol extracts of Black Pepper nigrum L. on the weight white rats females (body weight in gram).

Groups Treatment	G1	G2	G3
T1	25.3±3.12	29.11±1.43	21.22±1.33
T2	23.91±2.23	24.88±1.61	23.4±2.21

G1= Concentration 50 mg/ kg

T1= Ethyl acetate

G2= Concentration 100 mg/ kg

T2= Ethylic alcohol

G3= control group

Table (2) referred to a significant decrease in the ovaries weight after being treated with Ethyl acetate of Pepper Capsicum spp. of both concentrations in comparison with control group, where it reached (20.85±2.23), and (11.14±1.99) respectively, the concentration of (100) mg/ kg showed a significant decreased higher than ovaries weights, where it reached (11.14±1.99) of Ethyl acetate and (18.99±2.05) of Ethylic alcohol extract, the results have showed a significant increase in uterus weights after being treated with Ethyl acetate and Ethylic alcohol of Pepper Capsicum spp. of both concentrations in comparison with control group, where it reached (740.44±60.33), and (497.5±62.55) respectively.

Table (2): The effect of interference of various concentrations of Ethyl acetate and Ethylic alcohol extracts of Pepper Capsicum spp. on the weights of ovaries and uterus white rats females (organ mg/100 g of body weight).

Groups Treatment		G1	G2	G3
T1	Weight of ovary	20.85±2.23	11.14±1.99	39.55±2.01
	Weight of uterus	650.51±55.16	740.44±60.33	312.77±45.99
T2	Weight of ovary	28.78± 2.97	18.99±2.05	43.65±3.11
	Weight of uterus	497.5±62.55	622.70±58.81	324.86±2.34

G1=Concentration50mg/kg

G2=Concentration100mg/kg

G3= control group

T1= Ethyl acetate

T2= Ethylic alcohol

The results of table (3) referred to a significant increase of uterus layers thickness of both concentrations in comparison with control group, where it reached (199.65), and 325.23) of uterus muscle layer of both extracts respectively, the concentration of (100) mg/ kg showed a significant increase higher in uterus muscle thickness of both extracts, where it reached (275.78 and 391.44) of uterus muscle thickness of both extracts respectively, Ethyl acetate showed a higher activity in the increase of uterus layers thickness (uterus muscle and endometrium) in comparison with Ethylic alcohol extract as the results of statistical analysis results have showed the existed significant differences.

Table (3): The effect of interference of various concentrations of Ethyl acetate and Ethylic alcohol extracts of Black Pepper nigrum L. on uterus layer thickness of white rats females (layers thickness in micrometer).

Groups Treatment		G1	G2	G3
T1	uterus muscle	199.65±18.48	275.78±19.22	118.53±18.12
	uterus endometrium	325.23±14.23	391.44±15.33	230.56±17.82
T2	uterus muscle	175.59± 18.97	255.43±16.77	123.92±20.16
	uterus endometrium	298.25±20.59	375.87±18.81	234.86±20.34

G1= Concentration 50 mg/ kg

T1= Ethyl acetate

G2= Concentration 100 mg/ kg

T2= Ethylic alcohol

G3= control group

Discussion

The results of current study have showed a significant increase in body weight of rats females being treated only with Ethyl acetate for injection period of 10 , 15 days respectively in comparison with control group.

This result could be interpreted on a base of plant having of turbine, alkaline and Vinulini materials working on increase of body metabolization through motivating the production of proteins in addition to motivation to make cholesterol inside the body, their results matched with what has been mentioned by (4). The estrogenic activity of plant could be the reason behind the increase in the total body weight and that was assured by (5,6).

Ethylic alcohol extract has showed a decrease in ovaries weights in comparison with Ethylic alcohol extract, the reason is that Ethyl acetate helps to extract components with medium polarization which could have the main reason in the above mentioned decrease, these results matched but with the difference of active significant with hat has been mentioned by (7). The significant decrease in ovaries weights after being treated with both extracts of all concentrations due to the effect of extract in lowering of follicular hormone level, this hormone is important in developing of ovarian follicle reaching to Graffian follicle and that was proved by the current study outcomes, where significant decrease has been found in hormone level (8).

A composite called Myostatin was found separated from Pepper Capsicum spp. has an effect on increase the weight of uterus by its important effect on uterus muscle layer, this result matched with what was mentioned by (8,9).

The significant increase in ovaries weights because of being treated with the extract in both types Ethyl acetate and of Ethylic alcohol has been interpreted on the base that Pepper Capsicum spp. are components similar to Phytoestrogens, one of the most important components is Piperine, these significant s with all they have of estrogenic activity will lead to an increase in the thickness of uterus layers thickness by increasing the amount of Actin Protein and Myosin, these results matched many researchers' results who worked in field of plant extracts, they found the same effect but unlike active significant (10).

It became clear from the results of current study an existence of significant decrease in the numbers of primary and secondary and Graffian's ovarian follicles. This result could be mainly as a result of extract role in decreasing of follicular hormone and lutenizing hormone through decreasing of pituitary body motivation to secrete these hormones, it has found that Pepper Capsicum spp. has an obstructing role of these hormones from pituitary body(11), and that was proved by the results of current study, where a significant decrease has been found at the level of this hormones which was mentioned by many studies done on Pepper Capsicum spp. as a pregnancy preventive plant or on other plant extracts proved their roles as pregnancy preventive plants (12).

Estrogenic content of Pepper Capsicum spp. assured by researches, specially Piperine may work to change hormonal balance inside the body(13) in a way reflecting follicular hormone levels in serum as a result of obstructing its secretion from pituitary axis, which been mentioned by (14).

Many researches in field of plant extracts mentioned that some of extracts including Pepper Capsicum spp. have a role in increase of steroids creation process inside the body and in ovaries in particular in addition to their estrogenic activity which may reflect this effect on depressive–pituitary axis (15,16). Pepper Capsicum spp. extract may work on deterioration events in the period of estrus rotation specially, in the periods of proestrus and the period of diestrus as a result of the effect of estrogen and progesterone hormones respectively(17), this effect reflects on the developing of follicles development and metabolism process in lab rats, this what has been noted in a study made (18).

Decrease in numbers of ovarian follicles and Graffian's follicles may explain to the progestin activity, this activity may work on making of deterioration in lutenizing hormone balance through the decreasing of secretion from pituitary body which was noted through many researches which have progestonic effect(19)

The increase in uterus muscle layer thickness and endometrium is mainly due to plant composites with estrogenic activity in their activity is similar to estrogen hormone, these composites are exist in more than a plant, researches assured their roles in increasing of uterus layer thickness through motivating of existing muscles inside uterus, especially increasing of shrank proteins, Aktine and Myosine proteins of uterus layer, many researchers in field of plant extracts confirmed their role in increasing of uterus muscle layer thickness and endometrium, but different than active significant (20,21,22).

Through the following up of females injected with (50 , 100 mg / kg) respectively, for (12 days) hasn't based on the existence of sperm in the vagina swab before a time period, no evidence shows pregnancy in females through the following up for a period of (30) days in comparison with control group, we haven't obtained any fetuses in females treated with both extracts Ethyl acetate, significant decrease in the results of our study in the numbers of primary and secondary ovarian follicles and Graffian follicles may represent one of the main reasons behind no fetuses or decrease of their numbers as a result of those treated with extract. Pepper Capsicum spp. may be with an ability to prevent growing of fertile ovum inside uterus which may give an explanation to the existence of sperms inside vaginal swab and no signs existed refer to pregnancy in rat females(23,24,25).

Many researches have showed that estrous rotation deterioration has an effect in deterioration of some hormones responsible for developing of follicles and metabolism events, especially follicular hormone and lutenizing hormone which reflected on the numbers of fetuses and no pregnancy making, which our current study showed, it became clear that there is a significant decrease in the both mentioned hormones in comparison with control group (26).

No pregnancy the could explain that plant has the power to kill sperms inside vagina and not reaching to the ovum in order to make fertility. Extract may work on changing of uterus endometrium layer especially in its secretion activity through the effect in the numbers of uterus glands existed in, which may cause non providing of fetuses with the efficient food in order to it grow and growing inside this layer which could cause not to develop, completion and the decrease in the number of fetuses at the final outcome (27,28) .

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