The contraceptive effects of ethyl alcohol and ethyl acetate extract of Hibiscus rosa sinensis in swiss femal albino experimental mice .

Sarmad nory gany M.B.CH.B; MS.c pharmacology.
Dep. of Pharmacology,college of medicine, university of kufa12

الخلاصة :
اجر ت [a]ذه الدراسة للتقصي عن الفعال ة البا يولوج ة للمستخلص الكحولي الاث لي وخلات الاث ل لنبات ورد الختمة Hibiscus rosa sinensis ودوره كمانع للحمل في اناث الفئران السو سر ة البـ ضاء mus musculus من سلالة (Balb /C) وبواقع (٧٢) فأرا ، وقسمت الاناث الى ثلاثة مجام ع واعطـ ت التركـ ز ن (١٢٥، ٢٥٠) ملغم /كغم لكلا المستخلصـ ن وقورنت مع مجموعة السـ طرة ولثلاث مدد حقن (١٢,٨,٤) يوماً.
وقد اظ ـرت نتائج الدراسة ما لي :-

- وجود ز ادة معنو ة (P<0.05) في وزن الجسم الكلي للاناث المعاملة بمستخلص خلات الاث ل والكحول الاث لي مقارنة مع مجموعة السـ طرة
- وجود انخفاض معنوي (P<0.05) في اوزان المبا ض وز ادة معنو ة في اوزان الارحام للاناث المعاملة بكلا التركـ ز ن ولكلا المستخلصـ ن مقارنة مع مجموعة السـ طرةوكان مستخلص خلات الاث ل [a]و الاكثر فعال ة مقارنة مع المستخلص الاخر.

Abstract :
The aim of this experiment to study the biological effects of ethlyl alcohol and ethyl acetate extract of Hibiscus rosa sinensis and its contraceptive role in 72 female experimental mice .
The female mice divided into 3 groups and were treated with (125 , 250 mg /kg) of each extract for three periods (4,8,12) days .
The study show significant increase (p<0.05) in the total body weight in female mice treated ethyl alcohol , while ethyl acetate extract showed no significant effect in comparison with control groups , also the study show significant decrease(p<0.05) in ovarian weight and significant increase (p<0.05) in uterine weight in female mice treated with both concentration of Ethyl alcohol and Ethyl acetate extracts in comparison with control groups, the ethyl acetate was more potent than other extract .

Introduction :

Hibiscus rosa sinensis known as china rose and shoe flower , the flower are large generally red in the original varieties[(1)] regarded as a most common plant widely used in the medicine , and its therapeutic role in many diseases especially those affecting gastrointestinal tracts was confirmed [(2,3)] .

This plant was used as contraceptive since 8th century and Bhavamishra mentioned it in its book as the most important contraceptive plant , and the women must not be taking this plant in the period of menstruation , because of inhibiting role of this plant on ovulation and maturation of oocytes to Graffain follicles . Richard 1980 [(4)] found the estrogenic and progesteronic activities of this plant in addition to disturbances of astrocytes cycle in female mice , nowadays infertility role of this plant in female mice was confirmed [(5,6)] .

Materials and Methods :

1- Preparation of plant Extracts

The organic extracts of Hibiscus rosa sinensis Ethyl alcohol and Ethyl acetate were prepared using Ladd et al 1978 [7] method , 20gm was taken from dried substance of flowers then after ingredients were extracted respectively by soxholate instrument by adding 200ml for each solvent separately starting by ethyl acetate than ethyl alcohol for 24 hour , then the extracted ingredients concentrated in evaporating rotator (40-45c°), thenafter dissolve 29m of each solubility extracts in 10 ml distilled water to get stock solution (0.2 gm/ml) and repeated this process to get available active substances

2- Preparation of experimental animals :

This study involves (72) female albino swiss mice their age range (8-12 week) and their weight (20-30 gm) and the animals were entered the animal house and put them in a plastic cages with special reticulated cover and the animal were put under experimental conditions with Temp . (21-30 c°) and constant light for 13 hours and 11 dark hours .

3- Dissecting the animal :

The female mice were anesthetized by chloroform thenafter were dissected at 5^{th} day , regarding the animal groups which were injected with different concentration of both extracts , while the females which were injected for 8 days were dissected at 9^{th} day, and those were injected for 12 days were dissected at 13^{th} day of experiment .
The abdominal cavity were opened , and different organs of female reproductive system (ovaries and uterus) were extracted , put them in formalin solution (10 %) , the sticky sebaceous substances were removed and dried by filter paper .

4- Measurement of total body weight and genital organs weight

The female mice weight were measured before and after the end of experiment at (4,8,12) days by simple balance and compare with control groups .
The weight of genital organs (ovaries and uterus) which were extracted after killing process and dissection , measured using sensitive balance.

Results :

Result statistically analyzed using factorial experiments with completely randomized design using least significant difference (L.S.D) under the level 0.05 and standered error[8] .

The effect of different concentration of both ethly acetate and alcohol ethly extract of Hibiscus rosa sinensis on total body weight in female experimental mice (body weight in gram).

Table -1- show significant increase ($p<0.05$) in total body weight after treatment with different concentration of ethyl alcohol and ethyl acetate extract of Hibiscus rosa sinensis in comparison with control groups, 250 mg/kg causes significant increase in total body weight (28.654) .

2-Effect of both ethyl acetate and ethyl alcohol extract of 'Hibiscus rosa sinensis on total body weight of female mice in different periods of injection .

Table -2- shows significant increase ($p<0.05$) in total body weight after treatment with ethyl acetate extract of Hibiscus rosa sinensis at (4,8,12) days , also result show no significant increase in total body weight after treatment with ethyl alcohol extract of Hibiscus rosa sinensis for all days .

Table (1)

The effect of different concentration of both ethly acetate and alcohol ethly of Hibiscus rosa sinensis on total body weight in female experimental mice (body weight in gram).

Ethyl alcohol extract (total body weight after treatment)	Ethyl acetate extract (total body weight after treatment)	Conc. mg/kg
mean ± standard error	mean ± standard error	
1.432 ± 26.232	2.603 ± 28.121	125
1.523 ± 26.354	1.654 ± 28.654	250
2.314 ± 23.652	1.301 ± 22.825	Control

Value of L.S.D. in total body weight=2.631

Table (2)

Effect of both ethyl acetate and ethyl alcohol extract of 'Hibiscus rosa sinensis on total body weight of female mice in different periods of injection .

Ethyl alcohol extract (total body weight after treatment)	Ethyl acetate extract (total body weight after treatment)	Periods of injection
mean ± standard error	mean ± standard error	
2.101 ± 23.231	2.654 ± 28.101	4 days
1.133 ± 23.631	2.130 ± 29.643	8 days
1.893 ± 23.681	1.543 ± 29.656	12 days

Value of L.S.D. in total body weight=3.102

3-The effect of different concentration of both Ethyl acetate and alcohol Ethyl of Hibiscus rosa sinensis on ovaries and uteri weight in female experimental mice .

Table -3- show significant decrease ($p<0.05$) in ovaries weight after treatment with both concentration of ethyl acetate and alcohol Ethyl extract of Hibiscus rosa sinensis , concentration 250 mg/kg show significant decrease in ovaries weight of both extracts , also the table show significant increase in uteri weight after treatment with both Ethyl acetate and alcohol ethyl extract of Hibiscus rosa sinensis and for both concentration in comparison to control group .

4Effect of both ethyl acetate and ethyl alcohol extract of Hibiscus rosa sinensis on ovaries and uteri weight of female mice in different periods of injection .

Table -4- showed significant decrease ($p<0.05$) in ovaries weight after treatment with both ethyl acetate and alcohol ethyl extract of Hibiscus rosa sinensis . Ethyl acetate extract showed greater reduction in comparison to Ethyl alcohol extract, result also showed significant in uteri weight in all treated female mice .

Table (3)

The effect of different concentration of both ethyl acetate and alcohol ethyl extracts of Hibiscus rosa sinusis on ovaries and uteri weight in female experimental mice .

Ethyle alcohol extract	Ethyle alcohol extract	Ethyle acetate extract	Ethyle acetate extract	Conc mg/kg
Uterine weight	Ovarian weight	Uterine weight	Ovarian weight	
±standard error mean	±standard error mean	±standard error mean	±standard error mean	
59.813 ± 580.481	2.713 ± 32.431	60.561 ± 650.431	2.621 ± 23.462	125
60.321 ± 651.3	2.814 ± 17.531	61.731 ± 752.943	2.153 ± 12.521	250
53.421 ± 255.613	2.314 ± 47.321	± 252.671 52.252	2.653 ± 48.425	Con

L.S.D. value in ovarine weight=6.342
L.S.D. of value in uterine weight =138.654

Table (4)

Effect of both ethyl acetate and ethyl alcohol extract of Hibiscus rosa sinensis on ovaries and uteri weight of female mice in different periods of injection .

Ethyle alcohol extract	**Ethyle alcohol extract**	**Ethyle acetate extract**	**Ethyle acetate extract**	Period of injection
Uterine weight	**Ovarian weight**	**Uterine weight**	**Ovarian weight**	
mean ±standard error	±standard error mean	mean ±standard error	±standard error mean	
60.543 ± 432.918	2.815 ± 30.943	63.451 ± 540.100	2.116 ± 28.583	4th day
60.785 ± 476.814	2.951 ± 24.631	60.321 ± 580.111	2.563 ± 22.45	8th day
59.333 ± 521.431	2.651 ± 17.684	64.531 ± 690.432	2.614 ± 10.341	12th day

L.S.D. of value in ovarian weight = 8.953
L.S.D. of value in uterine weight =182.388

Discussion

The increment in total body weight in the albino swiss female mice treated ethyl alcohol and ethyl acetate extract of Hibiscus rosa sinensis could caused by phenolic and alkaloid contents of the plants which caused stimulation of protein , lipid synthesis including cholesterol and this result in concordance with result of AL shibbany 2005 [9] who studied the contraceptive role of black lettuces extract in experimental female mice .

The decrement in the ovaries weight could be due to a decrease in the FSH level which is important for follicular growth and maturation , this decrement could be caused by a progesteronic and estrogenic effect of the plants causing negative feed back inhibition of pituitary glands hormones[5,10] this result agree with Kholkute 1977[11,12] who found the inhibitory role of Hibiscus rosa sinensis on spermatogenesis and accessory reproductive organs.

The study showed that ethyl alcohol extract caused higher significant increment than ethyl acetate extract , this could be related to a role of ethyl alcohol in extraction the phenol compounds from original plant[13].

The significant increase in uteri weight in experimental female albino mice were attributed to role of phytoestrogenic content of the Hibiscus rosa sinensis plant which caused increase in a thickness of uterine layers[14,15].

Recommendation

further histopathological study for ovaries and uterus important to confirm contraceptive role of this plant .

1-Baza Mendonca, Luciana and Dos Anjos, Luiz (2005). Flowers in an urban area of southern Brazil. Brasileria de zoologia 22 (1): 51-59.

2-Batta SK and Shanthakumari G (1970) . Glossary of Indian medicinal plants . Indian J . Med ; 59 : 771 – 777 .

3-Fosters (1996) . Herbs for your health . Interweave press ; 91(1) 6 – 11

4-Richards JS (1980) . A treatise on medicinal uses of plants by Indian Tribes of Nevada . United States Department of agriculture ; 31 : 160 – 175 .

5-Chopra , R.N, Chopra, I.C , Hondak ,L , Kapur L.D , Chopra's indigenous(1992) . Drug of India ,Academic publisher . Calcutta , 1982 , p619.

6-Murthy DRK (1996) . Antifertility activities of Hibiscus rosa sinensis in female mice , Submitted to Gulbarga University ; 68 : 82– 89 .

7-Ladd JL , Jacobson M and Buriffim C (1978) . Beetles extracts promneem tree as feeding deterrents . J . Econ . Entomol ; 71 : 803 – 810.

8-Dani WW (1983) : Biostatistics . A foundation for analysis in the health sciences . Dani ed John Wiley andSons,NewYork

9-Alshibbany (2005). Effect of ethyl alcohol and ethyl acetate extact of black lettuce in infertility of female experimental mice.Ms.c thesis, college of science, university of kufa.

10-Blumenthal, M(Ed.)(1998): The complete German commission Emonographs :therapeutic Guide to Herbal medicines American Botanical council, Austin, Tx .

11-Kholkute SD (1977) . Effect of Hibiscus rosa sinensis onspermatogenesis and accessory reproductive organs in rats . Plant Med ; 31 : 127 – 130

12-Vasudera,(1998): post. Coital antifertility Activity of Hibiscus rosa-sineusis Linn, rootsGuru Jambheshwar unverisity of science and technology 09-30.

13- Yaginawa TR , Lzumi H , Yasimi T , Arai M , KawabetaR Fujmori O and Arakwa M(1992) Effect of traditional herbalmedicine on serum testosterone level and it inductionof regular ovulation in hyperandrogenic and oligomenorrheic women Acta obestGynecJapn;34:939–949 .

14-Dhawan S and Kambj R (1982). Effect of Hibiscus rosa sinensis .flower on the fertility of female mice . plant . Med ; 62:82- 90.

15- Kapoor M , Gary SK and Mathur VS (1979) . Quantitative clinical Chemistry . Indian J . Med ; 62 : 122 – 145 .