Effect of *Citrullus colocynthis* on some physiological aspects of female reproductive system in mice as a model for mammals

تأثير استخدام المستخلص الكحولي للحنظل على بعض المعايير الوظيفية للجهاز التناسلي لإناث الفئران كموديل للثدائن

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Abstract

The study was design to investigate the effect of oral administration of *Citrullus colocynthis* (CC) on some physiological and histological characters of female reproductive organs in mature mice of Swiss albino strain as a model for mammals. The animals were divided into three groups each contains 8 animals. Group one was treated with 6 mg/ Kg body weight BW/ day of alcoholic extract of CC, group two was treated with 9.6 mg/ Kg BW/day of alcoholic extract of CC and group three served as control throughout eight weeks period. The results of the study showed no significant ($P>0.05$) difference in the ovarian and uterine weight among groups. Significant ($P<0.05$) differences were shown in ovarian activity in control and group one compared to group two. The number of corpura lutea was highly significant ($P<0.01$) improved in group one than that of control group. The number of pregnant females was 50% in group one and control group. However the females treated with 6 mg/ Kg BW/ day of alcoholic extract of CC were gave a significant ($P<0.01$) results in the number of litters when compared with control group. It is concluded from the present research that treating with limiting dose of CC may accelerated the action of ovarian activity and resulted in improvement in the number of litters.

المستخلص

صممت الدراسة لبحث تأثير التجريع الفموي للمستخلص الكحولي لنبات الحنظل على بعض الخواص الفسلجيه و النسيجية للأعضاء التناسلية الانثويه للفئران الناضجة كموديل للثدائن . قسمت حيوانات التجربة إلى ثلاثة مجاميع كل مجموعه تحوي ثمان حيوانات جرعت ألمجموعه الأولى بالمستخلص الكحولي لماده الحنظل بتركيز 6 ملغم\كلغم من وزن الجسم\يوم . ألمجموعه الثانية عوملت بالمستخلص الكحولي لماده الحنظل بتركيز9,6 ملغم\كلغم من وزن الجسم \يوم أما ألمجموعه الثالثة أعطيت ماء فقط واعتبرت مجموعه سيطرة . استمر التجريع لمده ثمانية أسابيع . لم تظهر نتائج الدراسة فروقات معنوية ($P>0.05$) في أوزان الأعضاء التناسلية (المبايض والرحم) بين مجاميع التجربة . بينت النتائج فروقات معنوية ($P<0.05$) فيما يخص نشاط المبايض في مجموعتي السيطرة ومجموعة المعامله الأولى مقارنة مع مجموعه المعامله الثانية .

Key word: *Citrullus colocynthis*, reproductive organs, female mice, litters

أظهرت مجموعه المعامله الأولى فرق معنوي عالي (P<0.01) في أعداد الأجسام الصفراء مقارنة مع مجموعه السيطرة . نسبه الحمل في مجموعه المعامله الأولى و مجموعه السيطرة كانت 50% بينما الإناث المعامله بجرعة 6 ملغم\كلغم من وزن الجسم\يوم من المستخلص الكحولي لماده الحنظل سجلت أفضل فرق معنوي (P<0.01) بالنسبة لإعداد المواليد مقارنة مع مجموعه السيطرة . نستنتج من الدراسة الحالية إن المعامله بجرع محدودة من ماده الحنظل قد يزيد من النشاط المبيضي الذي ينتج عنه زيادة في أعداد المواليد الحية .

Introduction

Nature has been a source of medicinal agents for thousands of years, and an impressive number of modern drugs have been isolated from natural sources, many based on their use in traditional medicine [1]. *Citrullus colocynthis*, a well recognized plant in the traditional medicine of the natural order Cucurbitaceae, locally known as Handal that grows naturally in the western of Iraqi desert and in many other tropical and subtropical countries. Its fruit has been recommended for indigestion and diabetic people in traditional medicine [2, 3, 4], and was used by people as a pugative, antidiabetic and insecticide [5, 6]. The fruits are useful in tumors, ascitis, leucoderma, ulcers, asthma, bronchitis, urethrorrhea, jaundice, dyspepsia, constipations, elephantiasis, tubercular glands of the neck and splenomegaly [3, 7]. The plant can induce insulinotropic [2] and mild immunostimulating effects [8]; an unripe fruit cooked in hot sand is used to treat blennorrhagia (gonorrhea) in man [9]. Herbal derived substances remain the basis for a large proportion of commercial medications used today for the treatment of heart disease, high blood pressure, pain, asthma and other illnesses [10] as well as traditional Chinese herbal medicine also has a long history of use for infertility, one case report has linked use of a Chinese herbal product with reversible ovarian failure [11]. There are very few studies regarding the therapeutic effect of CC on several body systems. Therefore, the aim of this study is to find out the effect of *Citrullus colocynthis* on the female reproductive organs in mature mice as a model for mammals.

Materials & Method

Mature female mice of Swiss albino strain with an average of 28 ٣0 gm body weight (B.W) were kept in an air-conditioned room at (25±2) ℃ with light period of 13±2 hours. The mice were kept at least two weeks for adaptation. During this period abnormal and sick mice were excluded from the experiment. Twenty four mature female mice were divided into three groups each one involved eight animals. *Citrullus colocynthis* extract in a dose of 6 mg/ Kg.BW/day and 9.6 mg/ Kg.BW/day were orally administrated with drinking water to the female mice of group one and group two, respectively for eight weeks. The third group allowed drinking tap water only as control group. Four animals from each group were killed by cervical dislocation; the reproductive organs (ovaries and uterus) immediately freed from adipose tissue under dissecting microscope (VMF 4X, Japan) using fine surgical scissors and dried by filter paper then weight by an electronic precision balance (Sartorius, Switzerland). Histopathological sectioning of ovaries was done according to [12]. Other four females from each group were mated with two mature fertilized male mice. The number of litters was recorded.

Statistical analysis

Data were analyzed using statistical analysis system –SAS (2001) to study the effect of treatments in difference traits. Depending on the nature of data studied (t – test and F-test) through this program. When the results of F-test show a significant difference, least significant difference (LSD) was done to compare the significant difference between means [13].

Results

The results of this investigation revealed no significant (P>0.05) difference in the ovarian and uterine weight among groups Table (1). Significant (P<0.05) differences were shown in ovarian folliculogeneses in control and group one treated with 6 mg/ Kg BW/day of alcoholic extract of CC compared to group two treated with 9.6 mg/ Kg BW/day of alcoholic extract of CC. Best significant (P<0.05) improvement was shown in group one compared to control group in the numbers of corpura lutea as shown in Table (2). Pregnancy rate was equally improved (50%) in both control and group one (6 mg/ Kg BW/day) versus (0%) group two (9.6 mg/ Kg BW/day), while best improvement in litters numbers was observed in treated group one (6 mg/ Kg BW /day) when compared with control group Table (3).

Table (1): Effect of treatments with *Citrullus colocynthis* on some female reproductive organs weight

Groups	Reproductive system organs	
	Ovary weight (mg/100gm body weight)	Uterine weight (mg/100gm body weight)
Control	23.23 ± 4.99	362.81 ± 155.12
T1 (6 mg/ Kg.BW /day)	24.35 ± 4.05	435.11 ± 227.51
T2 (9.6 mg/ Kg.BW/day)	22.10 ± 4.36	340.58 ± 140.56

Values expressed as mean ± SD

No. mice per group = 4

ns No significant differences among groups

Table (2): Morphological changes in the numbers and types of follicles in the ovaries after oral administration of *Citrullus colocynthis*.

Groups	Types of follicles				
	Primordial	Primary	Secondary	Tertiar	CL
Control	7.00 ± 1.41	2.00 ± 0.00	2.25 ± 0.50	2.75 ± 1.89	0.50 ± 0.05
T1 (6mg/ Kg.BW/day)	*7.62 ± 3.70	*6.12 ± 1.72	*2.42 ± 1.51	*2.42 ± 1.27	*4.14 ± 2.41
T2 (9.6mg/ Kg.BW/day)	2.75 ± 1.48	2.50 ± 0.53	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

Values expressed as mean ± SD

No. mice per group = 4

*** P<0.05 a significant difference from corresponding groups.**

Table (3): Effect of *Citrullus colocynthis* administration on Pregnancy percentage and litters numbers

Treatment	Pregnancy percentage	Litters number (Mean ± SD)
Control	50%	4 ± 1.58
T1 (6 mg/ Kg.BW /day)	50%	9± 2.23*
T2 (9.6 mg/Kg.BW/day)	0%	0 ± 0.00

No. mice per group = 4

*** P<0.05 a significant differences from corresponding groups.**

Discussion

In this investigation after 8 weeks of treatment, the ovarian and uterine weight has not significantly affected after administrated of mature female mice with *Citrullus colocynthis* extract in a dose of 6 mg/Kg.BW/day and 9.6 mg/ Kg.BW /day, respectively. Other study has been found that ingestion of *Citrullus colocynthis* for 4 weeks resulted in slight but insignificant reduction in female rats body as well as uterine weight. [14, 15]. Long term exposure of female rats to CC showed a decrease in ovarian weights and a decrease in viable fetus's number [15]. As well as CC may be advised for ovarian trouble and during menstruation, if the pain griping spasmodic, sharp and sever [16]. Histological study revealed that low dose of alcoholic extract of CC significantly enhanced folliculogeneses when compared with high dose. This finding may have resulted from that the CC containing a number of different amounts of fatty acids, namely, palmic acid, stearic, oleic and linoleic [17]. These fatty acids may play an important role in oocyte maturation, fertilization and preimplantation embryos [18]. However, the increases of the CC concentration to 9.6 mg/ Kg.BW/day result in a decrease in the number of follicles and corpora lutia [19]. Other research work investigate that CC causes a decrease in fertility rate in a dose dependent manner but it has not any effects on the number of fetuses [20]. Pregnancy rate as well as litters number significantly improved in group administrated with low dose, *Citrullus colocynthis* kernels contain 52.0% oil, 28.4% protein, 2.7% fiber, 3.6% ash, and 8.2% carbohydrate. They are good sources of essential amino acids, especially arginine, tryptophan and methionine, vitamins B1, B2, niacin, Selenium (Se), Calcium (Ca), Magnesium (Mg), Manganese (Mn), Potassium (K), Phosphorus (P), Iron (Fe), and Zinc (Zn) [21]. Each of these chemical components has a good effect on the body reversible infertility [22]. Former data found that CC could decrease fertility rate at effective dose (10-100mg/kg/day) [23]. Regarding reproductive system, *Citrullus colocynthis* reduces fertility rate at dose rate of (30, 60 and 120 mg/kg/day) in mice [20]. It has been reported that those plants which show contraceptive properties have saponins, alkaloids, and glycosides compounds [24], *Citrullus colocynthis* contains saponin, alkaloids and glycosides component [25]. On the other hand, toxic effect of this plant (seeds and leaves extracts) could be noticed with the administration of higher doses leading to death [26]. In conclusion treating with limiting dose of CC may accelerate the action of ovarian folliculogeneses and resulted in improvement of the litter's number. However, the increase of the CC concentration causes adverse effects on the fertilization potential of mature female.

References

1. Gragg, G.M. and Newman, D.J. 2001. Medicinal for the millennia. Ann.NY Acad. Sci., (953):3 -25.
2. Numila, R.; Gross, R.; Rchid, H.; Roye, M.; Manteghetti, M.; Petit,P. ;Tijane, R. G. and Sauvaire, Y. 2000. Insulinotropic effect of *Citrullus colocynthis* fruit extracts. Planta Med. 66(5):418 – 23.
3. Ageel, A.M.; Mossa, J.S.; Al-Yahya, M.A.; Tariq, M. and Al-Said, M.S. 1987. Plants Used in Saudi Folk Medicine. King Saud University Press, Riydah.

4. Aburjai, T.M.; Hudaib, R.; Tayyem, M.Y. and Qishawi, M. 2007. Ethnopharmacological survey of medicinal herbs in Jordan, the J. Ethnopharmacol. ; 110: 294-304.

5. Warrier, P. K.; Nambiar, V. P. K. and Ramankutty, C. 1995. Indian Medicinal Plants. Orient Longman Ltd., Madras.1-5.

6. Diwan, F. H.; Abdel-Hassan, I. A. and Mohammed, S. T. 2000. Effect of Saponin on mortality and and histopathological changes in mice. Eastern Mediterranean Health J. 6 (2-3):345-351.

7. Al-Ghaithi, F.; El-Ridi, M. R.; Adeghate, E. and Amiri, M. H. 2004. Biochemical effects of *Citrullus colocynthis* in normal and diabetic rats. Mol. Cell Biochem. 261(1-2): 143-9.

8. Bendeddou, D.; Lalaoui, K. Satta, D. 2003. Immunostimulating activity of the hot water-soluble polysaccharide extracts of Anacyclus Pyrethrum, Alpinia galanga and *Citrullus colocynthis*. *J Ethno-pharmacol*.88 (2-3):155-60.

9. Burkill, H. M. 1985. The useful plants of west tropical Africa, *Citrullus colocynthis*. J. Tropical Animal Health and Production.1(2):83-89.

10. Saad, B.; Azaizeh, H. and Said, O. 2005. Tradition and perspectives of Arab Herbal Medicine: A Review. Evid. Based. Complement. Alternat. Med. 2 (4): 475 – 479.

11. Edmonds, S.E.; and Montgomery, J.C. 2003. Reversible ovarian failure induced by a Chinese herbal medicine: Lei gong teng. BJOG, 110: 77 – 8.

12. Humason, G.L. 1997. Animal Tissue Techniques. 5th ed., the Johns Hopkins University Press, Baltimore and London, pp: 361 – 378.

13. Sorlie, D.G. 1995: Medical Biostatistic and Epidemiology: examination and board review. 1st ed., Appleton and Lang, Norwalk, Connecticut, pp: 47 – 88.

14. Shapira, Z. J.;Terkel, Y.E. gozi, A. N. and Freidman J. 1989. Abortifacient potential for the epigeal parts of *Artemisia herbalbaa*. J. Ethnopharmacol., 27:319-325.

15. Waliad, S.h.;Qazan, Motasen, M. Al masad and Hytham Deradka 2007. Short and long effects of CC L. on reproductive system and fertility in female spague-Dawley rats. Pakistan J. Biol.Sci., 10(16):2699-2703.

16. Moor,M. Colocynthis-*Citrullus colocynthis*. 2003. Ellingwood′s American Materia Medica, 1919. M.Moore (ed.).South School of Botanical Medicine Press, USA.

17. Yaniv, Z. E.; Shabelsky, E. and Schafferman, D. 1999.Colocynth: Potential arid land oilseed from an ancient cucurbit. In J. Janick (ed). ASHS Press. Alexandria. 2:257-261.

18. Sturmey, R.G; A Reis; Leese, H.J and McEvoy, T.G. 2009. Role of fatty acids in energy provision during Oocyte maturation and early embryo development. J. Reproduction in Domestic Animals. 44(3):50–58.

19. Al-Dujaily, S. S. 2006. Effect of *Citrullus colocynthis* on certain sperm functions and live birth in mice: experimental model for mammals. J. Babylon University.12 (3):552-556.

20. Dehghani, F.; Azizi, M.; Panjehshahin, M. R.; Talaei-Khozani, T. and Mesbah, F. 2008. Toxic effects of hydroalcoholic extract of Citrullus colocynthis on pregnant mice. Iranian J. Veterinary Research, 9 (1). Ser. No. 22: 44.

21. Akobundu, E. N. T.; Cherry, J. P. and Simmons, J. G. 1982. Chemical, functional, and nutritional properties of egusi (*Citrullus colocynthis*) seed protein products. J. Food Science. 47 (3):829–835.

22. Chaturvedi, M.; Mali, P. C. and Ansari, A. S. 2003. Induction of reversible antifertility with a crude ethanol extract of *Citrullus colocynthis* schrad fruit in male rats. Pharmacology. 68 (1):38-48.

23. Balev, P.; Nikolaev, K. and Dzhidzheva, V. 1977. Several biochemical indices of the serum of cows with abortions of a non-infections nature. Vet. Med. Nauki. 14: 57-62.

24. El-zzi, A.; Benie, T.; Thieulant, M. L. and Duval, J. 1990. Inhibitory effects of saponins from Tetrapleura tetraptera on the LH released by cultured rat pituitary cells. Planta Med. 56: 375-379.

25. Abdel-Hassan, I.A.; Abdel-Barry, J.A. and Tariq, M.S. 2000. The hypoglycaemic and anti-hyperglycaemic effect of *Citrullus colocynthis* fruit aqueous extract in normal and alloxan diabetic rabbits. J. Ethnopharmacol. 71:325-330.

26. Adam, S. E. I.; Alfarhan, A. H. and AlYahya, M.A. 2000. Effect of combined *Citrullus Colocynthis* and *Rhazya stricta* use in najdi Sheep. American J. Chinese Medicine.1 (1):3.