

## **Effects of *Achillea Millifolium* extract consumption by pregnant mice on pregnancy outcome and reproductive system of their female off spring**

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### **Abstract:**

**Background:** *Achillea millefolium* (Yarrow) is medicinal plant that is traditionally used against inflammatory and spasmodic gastrointestinal complaint.

**Objective:** To study the effect of the consumption of *Achillea* extract by pregnant mice on pregnancy out come and on the reproductive system of the female off spring.

**Materials & Methods:** 70 adult pregnant female mice at age of 40-45 days were used in this study. These 70 mice were divided equally into two groups: experimental group (G1) and control group (G2). The experimental group was given 1mg/kg body weight/1ml of D.W of *Achillea millefolium* extract orally during 3 weeks of pregnancy, while the control group was given 1ml of D.W orally alone and for the same period.

After delivery the number and weight of the litters were recorded. 40 days after delivery, the weight of the female reproductive system was estimated, histological sections were done for the ovaries and oviducts, their diameters were measured, in addition serum was taken from these mice and measurement of FSH, LH and E<sub>2</sub> was done for both groups.

**Results:** The results of the experimental group showed highly significant decrease in litter size and significant decrease their weight. In addition young females born from treated mothers showed significant decrease in the weight of reproductive system, diameter of ovaries and diameter of oviducts. Also, significant decrease was noticed in the level of FSH, LH and Estradiol level in the experimental group.

**Conclusion:** Consuming of *Achillea millifolium* extract during pregnancy will produce bad effect on the pregnancy out come in addition to bad effect on the reproductive system of the female offspring.

**Key words:** *Achillea millifolium*, reproductive organs, hormones.

## تأثير استهلاك مستخلص القيصوم من قبل الفئران الحوامل على نتائج الحمل والجهاز التكاثري للمواليد من الاناث

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### الخلاصة:

**خلفية الدراسة:** القيصوم (باري) هو نبات طبي استعمل تقليدياً ضد الالتهابات والتقلصات المعوية. **هدف الدراسة:** لدراسة تأثير استهلاك مستخلص القيصوم من قبل الامهات الحوامل على نتائج الحمل والجهاز التكاثري لاناث المواليد.

**طريقة العمل:** استعمل في هذه الدراسة سبعين فأرة حامل بعمر 40-45 يوماً. قسمت هذه الفئران الى مجموعتين متساويتين: مجموعة الاختبار ومجموعة السيطرة. اعطيت مجموعة الاختبار 1 ملغم /كغم من وزن الفأرة من مستخلص نبات القيصوم مذاباً في 1مل من الماء المقطر فمويًا ولمدة 3 اسابيع خلال الحمل، بينما اعطيت مجموعة السيطرة 1 مل من الماء المقطر فقط فمويًا ولنفس الفترة. بعد الولادة تم احتساب عدد واوزان المواليد للمجموعتين. بعد اربعين يوماً من الولادة تم قياس اوزان الجهاز التكاثري لاناث المواليد واخذت مقاطع نسيجية للمبايض والقنوات الناقلة للبييض وتم احتساب اقطارها. اضافة الى ذلك تم اخذ عينات دم منها وقيس فيها مستوى الهرمون المحفز للجريبات FSH والهرمون اللوتيني LH والاسترادايول E<sub>2</sub> للمجموعتين.

**النتائج:** اظهرت نتائج مجموعة الاختبار نقصاً معنوياً عالياً في عدد المواليد و نقصاً معنوياً في اوزانها. اضافة الى ذلك فإن اناث المواليد لمجموعة الاختبار اظهرن نقصاً معنوياً في وزن الجهاز التكاثري، قطر المبايض وقطر قنوات البييض. كذلك نقصاً معنوياً في مستوى الهرمون المحفز للجريبات FSH والهرمون اللوتيني LH والاسترادايول E<sub>2</sub>.

**الاستنتاج:** ان استهلاك مستخلص القيصوم خلال الحمل ينتج عنه تأثير سلبي على نتائج الحمل اضافة الى التأثير السلبي على الجهاز التكاثري لاناث المواليد.

### Introduction:

There is perhaps no function more important in life than reproduction of the species. The exposure of pregnant mothers to toxic chemicals as well as to drugs that can be prescribed during pregnancy can exert toxic effects on the mother which can also mean exposure of the fetus as a result of circulating blood through the Placenta (1).

The use of medicinal herbs among the general population gives rise to the possibility of therapeutic or toxic

effect in patients seeking conventional medical assistance (2). Different species from the *Achillea millefolium* L. (Asteraceae) aggregate are used in traditional European medicine against gastrointestinal and hepato-biliary disorders due to their spasmolytic and antimicrobial properties (3). The recommended human dose is 50 mg/kg/day (4). It is popularly known as "yarrow", and it is widely distributed medicinal plant that has

been used for over 3000 years (5). Popular indications of this specie include treatment of wounds, hemorrhage, headache, inflammation, pain, spasmodic diseases, flatulence, and dyspepsia (6).

A review on the chemical constituent of *Achillea millefolium* was published recently and the following compound classes were reported as metabolites: Treprenoids, lignans, flavonoids & amino acid derivatives (7). Flavonoids have been demonstrated to have both estrogenic and antiestrogenic activities (8). As a result, yarrow has been used as an abortifacient by some ethnic groups (9), as a contraceptive (10), a stimulant for uterine contractions and to cure amenorrhea (11).

### Materials & Methods:

Seventy mature females (Swiss Webster) mice of 40-46 days age were used in this study. They were put in control cage under standard condition of temperature and illumination cycle (12 hr dark and 12 hr light), and free access to water and food.

Each proestrus female mouse was mated with one male mouse overnight. The presence of sperm in an estrus vaginal smear in the next morning was taken as evidence of mating. The day of positive smear was designated as day 0. Then these mice were divided into two equal groups: experimental (G1) and

control (G2) groups. The experimental group was treated with 1gm/kg body weight of alcoholic extract *Achillea millefolium* dissolved in 1ml of distilled water orally for three weeks during pregnancy, while the control group was fed with distilled water only by the same volume and period that used with experimental group. The number and body weight of all litters of both groups were recorded. Forty days later (about puberty age), the female animals were sacrificed to get their reproductive system out, weighting of these organs was done for both groups, then ovaries and oviducts were taken, the fixed histological sections with thickness of 5  $\mu\text{m}$  were prepared using the routine histological technique (12), ovarian diameter, as well as the diameter of the oviduct were measured. In addition blood samples were taken to measure the levels of FSH, LH and Estradiol hormones.

### Results:

**1- Effect of *Achillea millefolium* on pregnancy outcome:** When comparison was done for the birth rate and weight of the litters in the experimental and control groups, highly significant ( $p < 0.01$ ) difference in the birth rate was noticed in the experimental group result and significant ( $p < 0.05$ ) difference in their weight in comparison to control group (table-1- ).

**Table (1): Effect of *Achillea Millifolium* on birth rate and body weight of the mice offspring.**

parameters	Experimental group(G1)	Control group (G2)
Birth rate	3.7 ** ±0.016	8.3 ±0.023
Body weight	15.4* ±0.06	18 ±0.08

\*significant ( $p < 0.05$ ) difference.

\* \*Highly significant ( $p < 0.01$ ) difference.

### 2- Effect of *Achillea Millifolium* on weight of reproductive organs:

A significant ( $p < 0.05$ ) difference in the weight of reproductive organs of

female offspring was noticed in the experimental group compared to control group (table-2).

**Table (2): Effect of *Achillea Millifolium* on the reproductive organs' weight of female offspring.**

Parameter	Experimental G1	Control group G2
Weight of reproductive system	3.1* ±0.03	4.2 ±0.04

\*Significant ( $p \leq 0.05$ ) difference.

### 3- Effect of *Achillea millifolium* on histology of reproductive organs of the mature offspring:

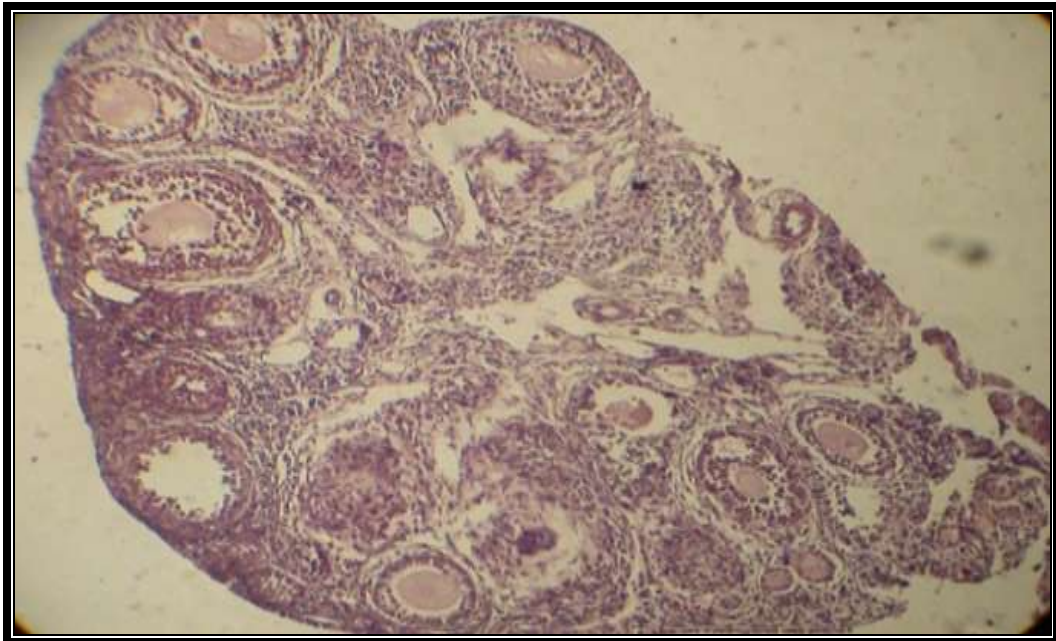
Table (3) show the changes in the histology of ovaries (Pic.1 and 2) and oviducts (Pic. 3and 4) of female offspring after administration of

*Achillea millifolium* to the mothers. Significant decrease ( $p < 0.05$ ) in the diameter of the ovaries and diameter of the oviducts were recorded in the experimental compared to control group.

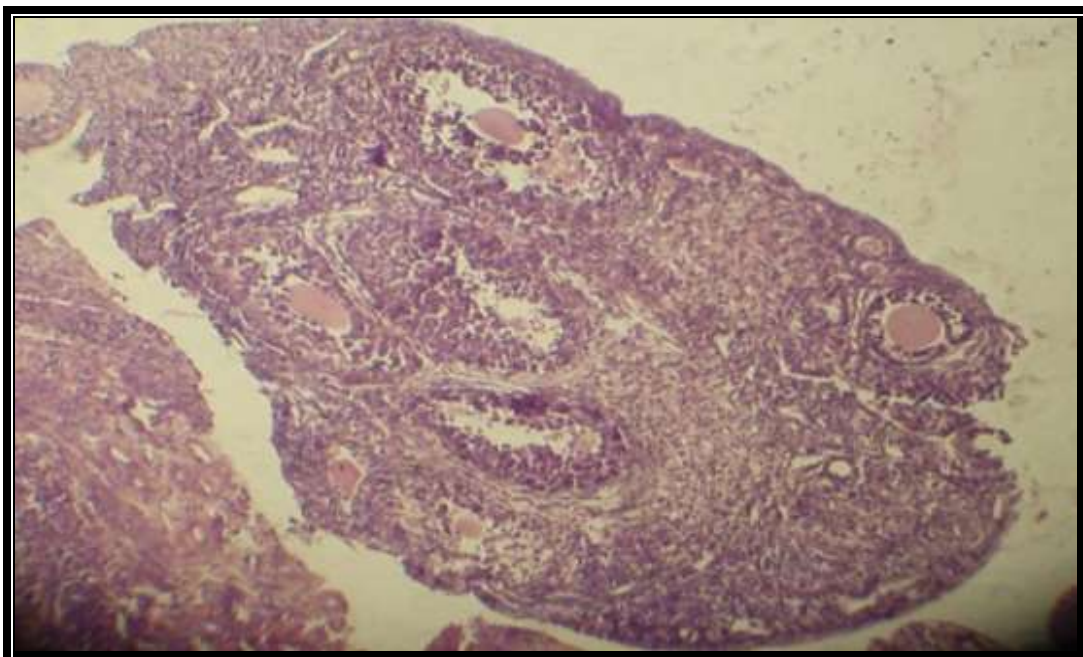
**Table (3): Effect of *Achillea Millifolium* on the diameter of ovary and oviduct of female offspring.**

Parameters	Experimental group G1	Control group G2
Diameter of ovary	18* ±0.07	20 ±0.1
Diameter of oviduct	7* ±0.01	9 ±0.02

\*Significant ( $p \leq 0.05$ ) difference.



**Figure (1):** The ovary of mature female mice from controlled group.



**Figure (2):** The ovary of mature female mice from the experimental group.



**Figure (3):** The oviduct of mature female mice from control group.



**Figure (4):** The oviduct of mature female mice from the experimental group.

**4- Effect of *Achillea millifolium* on reproductive hormones of the mature offspring:** When the results of the reproductive hormones (FSH, LH and Estradiol) of both groups

was compared, significant decrease ( $p < 0.05$ ) was found in the levels of these hormones in the experimental group compared to control group as shown in table (4).

**Table (4): The effect of *Achillea Millifolium* on reproductive hormones of female offspring.**

Parameters	Experimental group G1	Control group G2
FSH	3.3* ±0.02	4.8 ±0.03
LH	1.7* ±0.01	2.3 ±0.02
E <sub>2</sub>	5* ±0.023	6.3 ±0.03

\*Significant ( $p \leq 0.05$ ) difference.

### Discussion:

Herbal medicines have become a popular form of therapy in many countries. Even though they are often prompted as natural and therefore harmless, medicinal plants are by no means free from toxicity (13, 14). The present study was undertaken to evaluate the female reproductive toxicity of the aqueous extract of *A. millefolium* leaves.

In this study the litter size (birth rate) showed highly significant decrease for the mothers which received *Achillea* extract than those which were given D.W. only and this result does not agree with previous studies (11) and may assist the previous thoughts of *yarrow* as an abortifacient plant (15), and causing uterine contraction (16). The decrease in the birth rate may be explained to be due to the decrease in estradiol levels that may hamper ovulation, preparation of the reproductive tract for zygote implantation, and the subsequent maintenance of the pregnancy state (17, 18).

Also the present study showed a significant decrease in the body weight of the young females born from *achillea* extract treated mothers; this suggests that there is inadequate nutrient transfer across the placenta to the fetus (19). The spasmolytic action of some of the chemicals present in *yarrow* (Achillin, apigenin, and luteolin) may contribute to the decrease in fetal weight because they were shown to reduce gastrointestinal spasm and this reduction in motility may inhibit the absorption of nutrients into the maternal blood stream, reducing the availability of nutrients for placental transfer to the fetus, thus reducing the fetal weight (20, 21). These results agreed with that recorded in the previous studies (22, 23).

In addition the effect of *Achillea* extract was studied on ovaries and oviducts. Oviducts are dynamic organs that support gamete transport, maturation, fertilization, early embryonic growth and development, and the timely transport of embryos

for implantation in the uterus. Oviducts are targets of estradiol and progesterone produced in response to FSH and LH stimulation of ovaries (24). In this study significant decrease in the level of FSH, LH and Estradiol hormones are noticed. These hormonal imbalances may be caused by numerous chemical agents contained in plant extracts. Phytochemical screening has revealed many bioactive as well as toxic agents of plant extracts that can affect the regulation of oestrous cycle, conception and reproduction (25, 26). Alkaloids and flavonoids have been shown to reduce plasma concentrations of LH, estradiol and FSH (27, 28). It is possible that the extract might have exerted its effect on the anterior pituitary or the hypothalamus since the secretion of FSH is regulated by the gonadotropic releasing hormone secreted by the hypothalamus (29). Thus, the reduction in the serum concentration of estradiol observed in this study may be attributed to a decreased aromatase activity or substrate supplementation during estrogen synthesis (30). In addition it is well established that estradiol is directly responsible for the growth and development of reproductive organs (31), so that a significant decrease in diameter and weight of the ovaries in the experimental group was noticed.

#### **Conclusion:**

*Achillea millifolium* consumption in a dose of 1 gm during pregnancy cause significant decrease in number

and body weight of the offspring in addition to significant decrease weight of the reproductive organs, diameter of ovaries and oviducts in addition to its lowering effect on reproductive hormones FSH, LH and E<sub>2</sub>.

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#### **References:**

- 1- Kimberly P. Christina B. Chuck G. Dragana T. Jodi A. In utero effects of chemicals on reproductive tissues in females. *Toxicology and Applied Pharmacology*. 2004; 198: 111–131.
- 2- Bradley P. *British Herbal Compendium*. British Herbal Medicine Association. Dorset. 1992; 1: 227-229.
- 3- Willuhn G. *Millefolii herba*. In: Wichtl M. (Ed.), *Teedrogen und Phytopharmaka*. Wiss. Verl. Ges. Stuttgart. 2002 pp. 399–403.
- 4- Claire L. Boswell R. Helen E. Patricia D. Brown W. Preliminary screening study of reproductive outcomes after exposure to *Yarrow* in the Pregnant Rat. *Birth Defects Research (Part B)* 2003; 68:416–420.
- 5- Mitich L.W. *Intriguing World of Weeds: Yarrow –the herb of*



- Achilles*. Weed Technology. 1990; 4: 451–453.
- 6- Blumenthal M. Busse W.R. Goldberg A. *etal*. *Yarrow*. In: Herbal Medicine Expanded Commission E Monographs. Integrative Medicine Communications, Boston. 2000 Pp. 419– 423.
- 7- Si X. T. Zhang M. L. Shi Q.W. Chem Biodivers. 2006; 3:1163–1180.
- 8- Rachel S. Rosenberg Z. David J.A. Eleftherios P. Steroid hormone activity of flavonoids and related compounds. Breast Cancer Research and Treatment. 2000; 62: 35–49.
- 9- Devereux G. A study of abortion in primitive societies. New York: The Julian Press. 1955; Pp. 318.
- 10- Cambie R.C. Brewis A. A. Anti-fertility plants of the Pacific. Australia: CSIRO. 1997. Pp 60. `
- 11- Claire L. Boswell R. Helen E. Ritchie D. Patricia D. Brown W. Preliminary screening study of reproductive outcomes after exposure to *yarrow* in the pregnant rat. Birth Defects Research, (Part B). 2003; 68:416 -420.
- 12- Bancroft J. D. Steven A. Theory and practice of histological techniques. Churchill, Livingston, London, 2<sup>nd</sup> .ed. 1982 Pp110-111.
- 13- Blumenthal M. Busse W.R. Tyler V.E. *et al* The complete German commission E monographs therapeutic guide to herbal medicines. Integrative Medicine Communications, Boston. 2000;Pp 419–421.
- 14- Elvin Lewis M. Should we concern about herbal remedies. J Ethnopharmacol. 2001; 75: 141–64.
- 15- Devereux G. A study of abortion in primitive societies. New York: The Julian Press. 1955. pp 318.
- 16- Williamson G. The herbal medicine guide. Australia: Southwood Press. 1999. Pp 45.
- 17- Heasman L. Clarke L. Stephenson T. J. Symonds M. E. The influence of maternal nutrient restriction in early to mid-pregnancy on placental and fetal development in sheep. Proc Nutr Soc. 1999; 58:283–288.
- 18- Tewari J. P. Srivastava M.C. Bajpai J.L. Phytopharmacologic studies of *Achillea millefolium* linn. Indian J Med Sci. 1994; 28:331–336.
- 19- Boswell-Ruys C.L. Ritchie H.E. Brown-Woodman, P.D. Preliminary screening study of reproductive outcomes after exposure to *yarrow* in the pregnant rat. Birth Defects Research. Part B. Developmental and Reproductive Toxicology. 2003; 68: 416–420.
- 20- Hadley M.E. Hormones and female reproduction physiology. In:

Hadley ME, Ed. Endocrinology (5th ed.). Englewood Cliffs, NJ: Prentice Hall Inc. 2000. Pp 445 – 472.

21- Tewari J. P. Srivastava M. C. Bajpai J.L. Phytopharmacologic studies of *Achillea millefolium* linn. Indian J Med Sci .1994; 28:331–336.

22- Ota H. Kodama H. Fukuda J. Karube H. Tanaka T. Effects of the Kampo prescriptions and crude drugs on the steroidogenesis, particularly on aromatase activity in human granulosa cells. J. Fert Ster. 1995; 40: 355-361

23- Casanova M. You L. Gaido KW. *et al.* Developmental effects of dietary phytoestrogens in Sprague-Dawley rats and interactions of genistein and daidzein with rat estrogen receptors alpha and beta in vitro. Toxicol Sci. 1999; 51:236–44.

24- Pollow K. Inthraphuvasak J. Grill H. J. Manz B. Estradiol and progesterone binding components in the cytosol of normal human fallopian tubes. J Steroid Biochem. 1982; 16:429–435.

25- Benie T. Duval J. Thieulant M.L. Effects of some traditional plant extracts on rat oestrous cycle compared with clomid. Phytother Res. 2003; 17: 748-755.

26- Yakubu M. T. Akanji M. A. Oladiji A.T. Aphrodisiac potentials of the aqueous extract of *Fadogia agrestis* (Schweinf. Ex Hiern) stem

in male albino rats. Asian J Androl. 2005; 7: 399- 404.

27- Lauritzen C. Reuter H. D. Repges R. Bohnert K. J. Schmidt U. Treatment of premenstrual tension syndrome with *Vitex agnus castus* controlled double-blind study versus pyridoxine. Phytomedicine. 1997; 4: 183–189.

28- Bianco F. Basini G. Grasselli F. The plant alkaloid Sanguinarine affects swine granulosa cell activity. Reprod Toxicol. 2006; 21: 335-340.

29- Musa Y., Musba. A., Adenike O. *et al.* Johnston leaf extract on reproductive hormones of female rats Iranian Journal of Reproductive Medicine. 2008; Vol.6. No.3. pp: 149-155.

30- Hsia S. M. Yeh C. L. Kuo Y.H. Wang P. S. Chiang W. Effects of Adlay (*Coix lachryma-jobi* L. var. *ma-yuen* Stapf.) Hull extracts on the secretion of progesterone and estradiol in vivo and in vitro. Exptal Biol & Med. 2007; 232:1181- 1194.

31- Telefo P. B. Moundipa P.F. Tchana A. N. Tchouanguiep C. Mbiapo F. T. Effects of an aqueous extract of *Aloe buettneri*, *Justicia insularis*, *Hibiscus macranthus*, *Dicliptera verticillata* on some physiological and biochemical parameters of reproduction in immature female rats. J. Ethnopharmacol. 1998; 63: 193-200.