

Effect of alcoholic extract of *Achillea millefolium* consumption by pregnant mice on the uterus and reproductive hormones in the female offsprings

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Abstract:

Background

Achillea millefolium, (Asteraceae) is flowering plant which is popularly known as yarrow. It has been used in folk medicine to treat complaints such as inflammation, pain, wounds, hemorrhages and gastrointestinal disturbances.

Objectives

To study the histological changes in uterus and reproductive hormones in female mice delivered to mothers treated with *Achillea* extract during pregnancy.

Materials and Methods:

Seventy pregnant female mice at age of six weeks were used as a model for this study. The animals were divided into two groups: control (C) (35), and experimental (G) (35) groups. The experimental group was given 1.5 mg/kg alcohol extract of *Achillea millefolium* dissolved in 1 ml distilled water orally for the whole pregnancy, while the (C) was given distilled water only in the same amount and for the same period of time. The number and the body weight of the delivered offspring was recorded, in addition the endometrial thickness, the number and diameter of the uterine glands was measured in the histological sections at puberty. In addition evaluation of FSH, LH and E2 hormones levels were done.

Results

The results of this study showed highly significant ($p < 0.01$) decrease in the litter size of the experimental compared to the control group, as well as significant decrease ($p < 0.05$) in the weight of the offspring, the endometrial thickness, the number and diameter of the uterine glands in the (G) compared to the (C).

Conclusions

Consumption of low dose *Achillea millefolium* causes significant decrease in number and weight of the offspring, in addition to its effect on the uterus of the delivered female offspring.

Keywords: *Achillea millefolium*, Uterus, offspring.

Introduction

Plant-derived chemicals that influence endocrine activities in both humans and animals have received a great deal of attention due to their possible beneficial as well as adverse effects on human fertility (1). *Achillea millefolium* (yarrow) is a well-known species amongst the members of *Achillea*

(Asteraceae) (2). It is known as "Civanperçemi" and used in folk remedies as an appetizer, wound healer, diuretic, carminative or menstrual regulator (3), and it was used in traditional European medicine internally in the treatment of gastro-intestinal and hepato-biliary disorders and externally

in case of skin inflammations and for wound healing (4). More than 120 chemical compounds have been identified in this plant. (5). The main active compounds in yarrow are flavonoids (apigenin, rutin, luteolin, campherol) and essential oils (6). Flavonoids have been demonstrated *in vitro* and *in vivo* to have estrogenic as well as antiestrogenic activity (7, 8). These compounds have been shown *in vivo* to lengthen the follicular phase of the menstrual cycle (9), reduce urinary excretion of 17 β -estradiol, and favor 2-hydroxyestrone formation (10). Health hazards associated with long-term exposure to *Achillea millefolium* extracts are not well established. Despite the Food and Drug Administration (FDA) has classified the plant as nonpoisonous and has approved its utilization in alcoholic drinks (11). Some toxic effects had been reported after its use by humans and in animal experiments. Toxic effects in human include contact dermatitis (12), headaches and dizziness (13, 14). The recommended human dose is 50 mg/kg/day (15).

Aim of the study: To evaluate the effect of *Achillea millefolium* extract consumption during pregnancy on the uterus and reproductive hormones of the female offspring.

Materials and Methods

Seventy mature female mice were used in an age of 6 weeks as a model in this study. Mating was done. Estrus vaginal smear in the next morning was taken and presence of sperms in the smear considered as indication of mating. These mice were divided into two groups: Experimental group which consist of (35) mature mice and the control group which consist of (35) mice. These two groups were put under the same condition of temperature food and water supply. The experimental group was given 1.5 mg/kg (body weight) alcoholic extract of *Achillea millefolium* dissolved in 1ml of distilled water orally. The other group (control group) was given distilled water only in similar amount and route of administration and for the same period of time. After 3 weeks the number and weight of the offspring were recorded. When the female offspring reached the time of puberty they were sacrificed and blood samples were taken from them to measure FSH, LH and E2. In addition the uterus was taken from them and histopathological examination was done by (16) to measure the endometrial thickness, number and diameter of the uterine glands was done for both groups.

Results

1-Effect of *Achillea millefolium* on pregnancy outcome: The calculated results of this study showed highly significant ($p < 0.01$) decrease in the litter size, and significant decrease ($p < 0.05$) in the body weight of the offspring in the experimental group when compared to the control group. 2-Effect of *Achillea millefolium* on the uterine histological section of mature female offspring: The results of the present study showed significant decrease ($p < 0.05$) in the endometrial thickness, number and diameter of the

endometrial glands, as shown table, Figure (1,2).

3-Effect of *Achillea millefolium* on the gonadotrophic and estradiol hormones of the female offspring: Significant decrease ($p < 0.05$) in the level of FSH and LH, as well as E2 hormones in the experimental compared to control group

Discussion:

Yarrow *Achillea millefolium* is a plant which functions as a biological additive in cosmetic products and its extract was used in 65 cosmetic formulations. Sesquiterpene lactones, polyacetylenes, flavonoids and essential oil have been identified as components of *Achillea millefolium* (17). In the present study alcohol extract of this plant was used during the pregnancy period of the experimental mice. The results showed that the number of the live birth was less in the treated group compared to control group and this may be due to the effect of *Achillea* extract on the estradiol level, decreasing it and interfering with embryo implantation and maturation (18). This result does not agree with that of the previous studies (19). Another part of the study was the significant decrease in the body weight of the offspring in the treated group and this may be explained due to the spasmotic effect of *Achillea* plant that inhibits absorption of nutrients and subsequent decrease in nutrient transfer across the placenta and thus diminished fetal body weight (20) and this finding agreed with that of previous studies that high phytoestrogen diet will decrease fetal body weight (21).

The use of *Achillea millefolium* by pregnant mice showed that the endometrial thickness, the number and diameter of the endometrial glands were less in the female offspring of the experimental group which received *Achillea* extract compared to control group which received distilled water only. This result was similar to that of previous studies (22), and this may be explained to be due to lowering effect of flavonoids on estradiol level which is the hormone responsible for the growth of endometrium during follicular phase (23), but this result does not agree with that of Unfer et al that these estrogen compound will increase the endometrial thickness (24).

The levels of FSH, LH and E2 showed significant decrease in the offspring of the experimental group and this is because plants with estrogenic property can directly influence pituitary action by peripheral modulation of LH and FSH, decreasing secretion of these hormones and blocking ovulation (25). 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 (26). Consequently, such decrease in estradiol levels may hamper ovulation, preparation of the reproductive tract for zygote implantation, and the subsequent maintenance of the pregnancy state (27). Our findings contrast that of Ota et al. (28) and similar to that of Musa et al. (29).

Table 1: Effect of *Achillea millefolium* on the uterine histological section of mature female offspring

	Treated group	Control group
Birth rate	4.1 ±0.01	8.1 ±0.02
Body weight (g)	15.4 ±0.06	18 ±0.08
Number of endometrium gland	2 ±0.005	3 ±0.01
Endometrium thickness	2.6 ±0.007	3.4 ±0.01
Diameter of uterine gland	0.13 ±0.001	0.26 ±0.002
FSH (mlu/ml)	3.1 ±0.0	4.6 ±0.03
LH (mlu/ml)	1.4 ±0.023	2.2 ±0.03
E2 Pg/ml	4.8 ±0.03	6.2 ±0.04

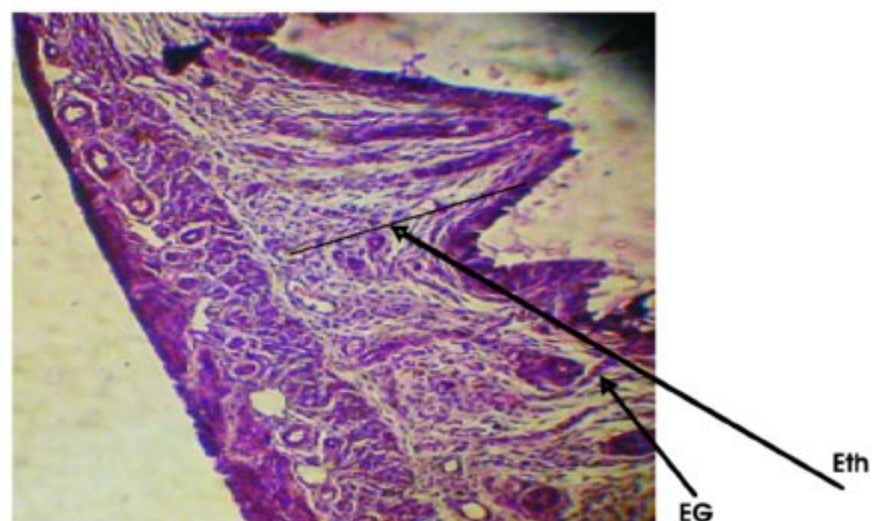


Figure 1: Longitudinal section in uterine horn of a mature female mouse (treated group), showing the endometrial glands diameter (EG), and endometrium thickness (Eth), (H&E, 40X)

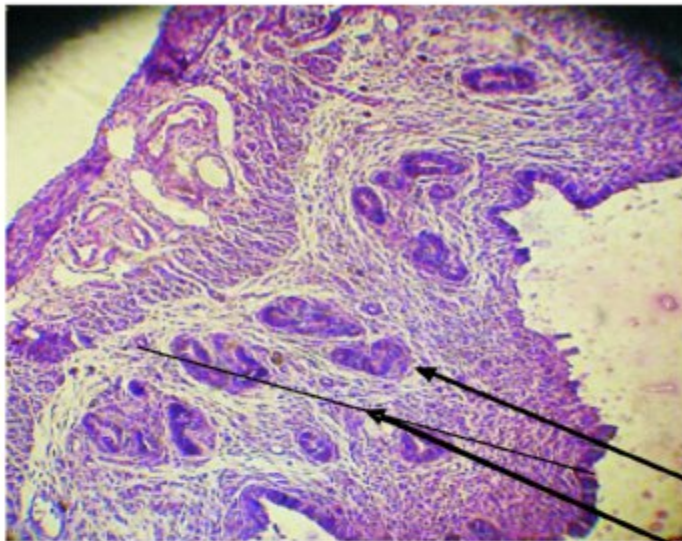


Figure 2: Longitudinal section in uterine horn of a mature female mouse (control group), showing the endometrial glands diameter (EG), and endometrium thickness (Eth) (H&E, 40X)

EG
Eth

References

- 1- Gamache PH and Acworth IN. Analysis of phytoestrogens and polyphenols in plasma, tissue and urine using HPLC with coulometric array detection. *Proc Soc Exp Biol Med.* 1998; 217: 274-280.
- 2- Chandler RF, Hooper SN and Harvey NJ. Ethnobotany and phytochemistry of yarrow. *Achillea millefolium*, Compositae. *Economic Botany.* 1982; 36: 203-215.
- 3- Baytop, T. (treatment with plants in Turkey). Istanbul University Publications 1999; 255: 40-176.
- 4- Willuhn G and Wichtl M. *Teedrogen und Phytopharmaka*, fourth ed.. Wiss. Verl.-Ges, Stuttgart. 2002. Pp. 399-403
- 5- Chandler R, Hooper S and Harvey M. *Compositae Econ Bot.* 1982; 36: 203-23.
- 6- Bimbirait 1 K, Ragainskien O, Maruka A and Komyova O. Comparison of the chemical composition of four yarrow (*Achillea millefolium* L.) morphotypes. *biologija.* 2008; 54 (3): 208-212
- 7- Wang T.T., Sathyamoorthy N. and Phang JM. Molecular effects of genistein on estrogen receptor mediated pathways. *Carcinogenesis.* 1996; 17: 271-275.
- 8- Hsieh CY, Santell RC, Haslam SZ and Helferich WG. Estrogenic effects of genistein on the growth of estrogen receptor-positive human breast cancer (MCF-7) cells in vitro and in vivo. *Cancer Res* 1998; 58 (19): 3833-3838.
- 9- Cassidy A, Bingham S. and Setchell K.D. Biological effects of a diet of soy protein rich in isoflavones on the menstrual cycle of premenopausal women. *Am J Clin Nutr.* 1994; 60: 333-340.
- 10- Xu X., Duncan A.M., Merz B.E. and Kurzer MS. Effects of soy isoflavones on estrogen and phytoestrogen metabolism in premenopausal women. *Cancer Epidemiol Biomarkers Prev.* 1998; 7: 1101-1108.
- 11- Duke JA. *Achillea millefolium* L. (Asteraceae): yarrow. In: *Handbook of Medicinal Herbs.* CRC Press, 1987. Pp. 9-10.
- 12- Hausen BM, Breuer J, Weglewski J and Rucker G. Alphaperoxyachifolid and other new sensitizing sesquiterpene lactones from yarrow (*Achillea millefolium* L. Compositae). *Contact Dermatitis.* 1991; 24: 274-280.
- 13- Alonso JR Milenrama. In: *Tratado de Fitomedicina: bases clínicas y farmacológicas.* Isis, Buenos Aires, 1998. Pp. 725-729
- 14- C'aceres A. *Plantas de Use Medicinal en Guatemala.* Universit'aria, Guatemala, 1999. Pp. 268-270.
- 15- Claire L, Boswell-Ruys, Helen E, Ritchie -Woodmann. PD Preliminary Screening Study of Reproductive Outcomes After Exposure to Yarrow in the Pregnant Rat. 2003; 68: 416-420
- 16- Bancroft JD and Steven. *Theory and practice of histological techniques*, 2nd ed., William clowei (Beccles) Limited Beccles and London ,Great Britain. 1982.
- 17- Hausen BM. A6 - year experience with compositae mix. *Am.J.Contact Dermatitis.* 1996; 7: 94-99.
- 18- Tewari JP, Srivastava MC and Bajpai JL. Phytopharmacologic studies of *Achillea millefolium* linn. *Indian J Med Sci.* 1994; 28: 331-336.
- 19- Claire L, Boswell-Ruys, Helen E, Ritchie, and Patricia D. Brown-Woodmann Preliminary. Screening Study of Reproductive Outcomes After Exposure to Yarrow in the Pregnant Rat Birth Defects Research. 2003; 68: 416-420.
- 20- Tewari JP, Srivastava MC, Bajpai JL. Phytopharmacologic studies of *Achillea millefolium* linn. *Indian J Med Sci.* 1994; 28: 331-336.
- 21- Rachel L, Kembra L, Jiude M, Julia A., Taylor F. Phytoestrogen Levels in Feed Increase Fetal Serum Estradiol Resulting in the "Fetal Estrogenization Syndrome" and Obesity in CD-1 Mice. *Environmental Health Perspectives.* 2008; 3: 116-322.
- 22- Yang J, Nakagawa H, Tsuta K. and Tsubura A. Influence of perinatal genistein exposure on the development of MNU-induced mammary carcinoma in female Sprague- Dawley rats. *Cancer Lett.* 2000; 149: 171-179.
- 23- Bianco F, Basini G and Grasselli F. The plant alkaloid Sanguinarine affects swine granulose cell activity. *Reprod Toxicol.* 2006; 21: 335-340.
- 24- Unfer V, Casini MA, Costabile L, Mignosa M, Gerli S. and Di Renzo GC. Endometrial effects of long-term treatment with phytoestrogens: a randomized, double-blind, placebo-controlled study. *Fertil Steril.* 2004; 82: 145-8.
- 25- Brinker F. Inhibition of endocrine function by botanical agents, Antigonadotropic activity. *Br J Phytother.* 1997; 4: 123-145.
- 26- Hsia SM, Yeh CL, Kuo YH, Wang PS and 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. *Exptl Biol & Med.* 2007; 232: 1181-1194.
- 27- Hadley ME. Hormones and female reproduction physiology. In: Hadley ME, Ed. *Endocrinology* 5th edition. Englewood Cliffs, NJ: Prentice Hall Inc. 2000. Pp. 445-472.
- 28- Ota H, Kodama H, Fukuda J, Karube H and Tanaka T. Effects of the Kampo prescriptions and crude drugs on the steroidogenesis, particularly on aromatase activity in human granulose cells. *Fertil Steril.* 1995; 40: 355-361.
- 29- Musa Y, Musbau A, Adenike O, AbdulWaheed O, Abdulfatai A, Monsurat Y, Bamidele O., Taofik S. and Moyosore A. Effect of *Cnidioscolous aconitifolius* (Miller) I.M. Johnston leaf extract on reproductive hormones of female rats Iranian J. *Reprod. Med.* 2008; (6)3: 149-155.