Effect of Achillea Millefolium Administration by Pregnant Mice on the Testes of their Offspring at Puberty

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Abstract:

To study the histological in testes and parameter of seminal fluid of male mice delivered to mothers treated with Achillea extract during pregnancy. In this research the effect of administration of alcoholic extract of Achillea mellefolium flowers on fertility indices, body weight and reproductive organs weight was evaluated in male mice.

60 mature female mice at age of six weeks (during pregnancy period) were used as a model for this study. The animals were divided into two groups, control group, and experimental animals. Animals in control group received (1 ml) of distilled water and experimental group received (1 ml/kg) alcohol extract of Achillea millifolium dissolved in 1ml distilled water orally for the whole pregnancy period. At the end of 3 weeks of the treatment period, fertility indices such as litter size, body weight, weight of testes and parameter of seminal fluid of the delivered of spring was recorded, in addition the thickness and diameter of semniferous tubules was measured in the histological sections, which were made for the testes of the male offspring when they reached the time of puberty .

The alterations observed of this study showed significant ($P < 0.05$) decrease in litter size, body weight, weight of testes and parameter of seminal fluid of the experimental compared to the control group, in addition significant ($P < 0.05$) decrease in the thickness and diameter of semineferous tubules of the experimental compared to the control group .

Introduction :

Medicinal planets, since times immemorial, have been used in virtually all cultures as source of medicine. [1]

The use of traditional medicine and medicinal plants in most developing countries, as a normative basis for maintenance of good health, has been widely observed. [2,3]

Achillea is a medicinal herb that has been used in popular medicine for its antimorrhagic. Healing and analgesic properties. [4,5]

It is native to Europe, North America, south Australia and Asia [6,7]. A millefoliuml. a dark green perennial with tough stems, 30-90 cm high with abundant, long, pinnate leaves and pink or white flowers.

The studies showed that Achillea millefolium and difference variety of Achillea sanatolina have an anti spermatogenic and degenerative changes on mice testes [8,9] .

Materials and methods

Plant material:

The flowers were homogenized by a blender and dried for 48 h at (40º C) air dried power (100 gram) was extracted by per colation at room temperature with (70%) hydroalcoholic solution. The extract was concentrated in vacuum desiccators and the residue was dissolved in (45%) hydroalcoholic solution. [1]

Animals and treatments:

(60) mature female mice at age of six week (during pregnancy period) were used as a model for this study. The animals were divided into two group: control (30) and experimental (30) animals .

The experimental group was given (1 mg/kg) alcohol extract of chillea millifolium dissolved in 1 ml distilled water orally for the whole pregnancy period, while the control group was given distilled water only in the same amount and for the same period of time.

Evaluation of parameters :

The mice were weighed at the beginning and at the end of the experiments. The mice were killed by cervical dislocation 24 h after the last dose. [9]

The letter size, body weight, weight of testes and parameter of seminal fluid of the delivered offspring was recorded, in addition the thickness and diameter of seminefeuros tubules was measured in the histological sections, which were made for the testes of the male offspring when the reached the time of puberty .

Histological studies :

The testes and epididimides were immediately fixed in formaldehyde solution (10%) and after tissue processing were embedded in paraffin wax and sectioned at (5 m) they were stained with hematoloxylin and eosin .

Morphological studies were evaluated by Olympus microscope at 40X magnifications . [2]

Statistical analysis:

We used SAS program (2004) in statistical analysis to study the effect of Achillea millefolium administration by pregnant mice on the testes of their off spring at puberty on fertility parameters of male mice and all result were expressed as means ± standard deviation. Difference between groups was considered to be significant at $p < 0.05$.

Result:

The results showed that administration of alcoholic extract of Achillea millefolium at doses of (1mg/kg)/day for 3 weeks caused significant ($p < 0.05$) decrease in the body weight, in addition, a significant decrease in testes weight and parameter of seminal fluid of the experimental compared to control group. In addition significant ($p < 0.05$) decrease the thickness and diameter of somineferous tubules of the experimental compared to the control group. (table1)

Table (1)

Effect of Achillea millefolium on the histological section of testes of mature male offspring

	Control group(C)	Experimental group(G)
Litter size	8.2 ± 0.03	6.3* ± 0.02
Body Weight offsprings At puberty	20.4 ± 0.1	17.7 ± 0.07
Weight of testes(mg)	0.56 ± 0.02	0.32 ± 0.01
Thickness of semineferous tubules(µm)	1.65 ± 0.03	1.34 ± 0.02
Diameter of semineferous tubules(µm)	14.3 ± 0. 01	11.9 ± 0. 07

* : $p < 0.05$, compared with control

Sperm motility and sperm viability: The results showed that administration of alcoholic extract of Achillea millefolium, caused no significant differences of sperm motility and sperm viability (table 2)

Table (2)

Effect of Achillea millefolium consumption by pregnant mice on the Seminal fluid in the male offspring

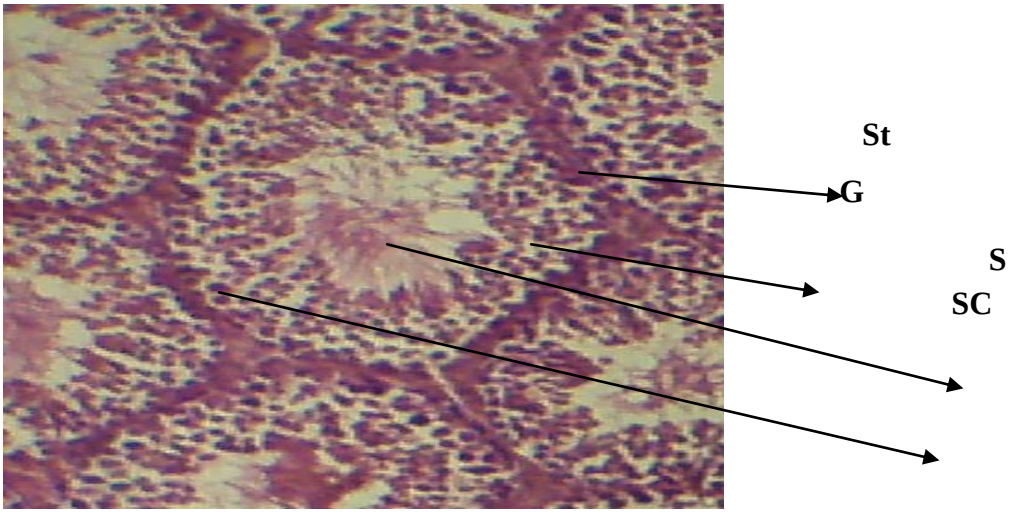
Seminal fluid	Control group(C)	Experimental group(G)
Sperm concentration million / ml	55.3 ± 0.07	40.2* ± 0.05
Sperm motility Grade A	24.9 ± 0.05	13.4 ± 0.03
Grade B	42.6 ± 0.04	35.8 ± 0.02
Grade C	25.4 ± 0.04	20.7 ± 0.06
Grade D	8 ± 0.03	30.1 ± 0.02
Abnormal morphology	62.3 ± 0. 14	66.1 ± 0. 1
viability	23.5 ± 0.03	20.4 ± 0.05

* : $p < 0.05$, compared with control

Histological findings:

The well organized semineferous tubules filled with germ cells and sertoli, with numerous sperm in the lumen. (figure 1) .

In treated animals (1mg/kg)/day orally for 3 weeks there were seen significant decline in the diameter and thickness of semineferous tubules with degenerative features of primordial germ cell, sloughish cells and absence of sperm from of semineferous tubules.



transverse section in testes of a mature male mouse (control group), showing the well organized semineferous tubules walls(st) filled with germ cells(g)and sertoli cells(sc),with numerous sperms in the lumen(sp) (H&E, 40X)

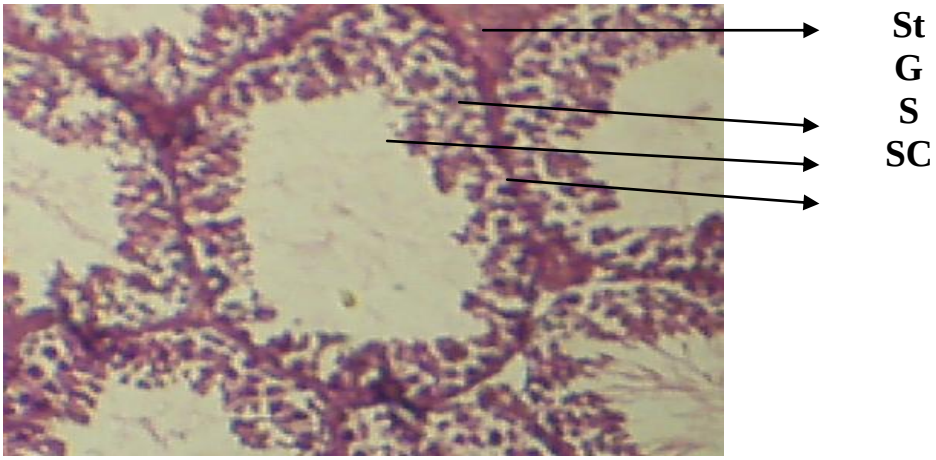


Fig (2)

transverse section in testes of a mature male mouse (treated group), showing significant decline in the diameter and thickness of semineferous tubules (st) with degenerative features of primordial germ cell (g),sloughing cells and absence of sperm from of semineferous tubules(st) (H&E, 40X)

Discussion:

Present investigation demonstrate that oral administration of alcoholic extract of Achillea millefolium (1mg/kg orally) for 3 weeks, cause a significant decrease in fertility parameters in male mice. [9]

Consuming of Achillea millefolium by pregnant female mice causes a significant decrease in the litter size, body weight, weight of testes and parameter of seminal fluid of the experimental compared to the control group, in addition significant decrease the thickness and diameter of semineferous tubules of the experimental compared to the control group.

The results of this study are similar to those of Montanari: etal. [8]

It was found that weight of testes in treated animals significantly decreases.

Also there was found an alteration in the spermatogenesis process, such as disorganized germ epithelium, degenerated and necrotic cells and reduction of germ epithelium.

These alterations were also reported with Achillea millefolium [8] , gossypol [10] and trypterygium Wilfordii: [11] , which are considered to be antispermatogenic agents.

The decrease in (testes weight / body weight) and epididymis weight may be attributed to increase level of damage on the testes tissue of experimental rates that showed in previous studies [8 , 9] .

The exact mechanism of these effects is not clear and might be due to substances present in Achillea millefolium extract, which leads to its anti fertility effects, but it may be due to presence of chemical composition of Achillea millefolium. As paradin [9] show that oral administration of alcoholic extract of Achillea millefolium, cause a significant degrease in fertility parameters in male rats .

In its chemical composition, literature describes the presence of essential oil with terpens (cineol, borneol, pinens, camphor, azulen) terpenics and sesquiterpenics derivatives, tannins, coumarins, resins, saponin, steroids, fatty acid, alkaloids and principles of bitter taste [6, 13 ,14]. It is reported the presence of flavonoids, apigenin, luteolin and its glycosiders, artemelin and rutin in the flowers and leaves [15 , 16] .

Sludies showed that apigenin (a flavonoid) was an effective inhibitor of aromatase (human estrogen synthetase) and 17 b-hydroxy steroid dehydrogenas activites in human placental microsomes [17].

In conclusion, alcoholic extract of Achillea millefolium by pregnant female mice causes a significant decrease in the litter size, body weight, weight of testes and parameter of seminal fluid of the experimeutal compared to the control group, in addition significant decrease the thickness and diameter of semineferous tubules of the experimental compared to the control group.

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تأثير استهلاك الفئران الحوامل للمستخلص الكحولي لنبات القيصوم على الخصى للمواليد الذكور

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الخلاصة

لدراسة التغيرات النسيجية في الخصى وقياس المعايير في السائل المنوي في ذكور الفئران المولودة من الاناث المعاملة بمستخلص القيصوم خلال فترة الحمل , في هذا البحث تم تقييم التأثيرات الناجمة عن اعطاء المستخلص الكحولي لنبات القيصوم للامهات والمتمثلة بقياس الخصوبة وزن الجسم وكذلك وزن الاعضاء التكاثرية في الذكور المولودة , استمرت التجربة لمدة ثلاثة اسابيع , استخدمت فيها (60) فأرة اناث ناضجة بعمر ستة اسابيع (خلال فترة العمل) التي تم اعتمادها كنموذج في هذا البحث .

قسمت الحيوانات الى مجموعتين , مجموعة السيطرة ومجموعة حيوانات التجربة , اعطيت مجموعة السيطرة (1ml) من المحلول الملحي الفسيولوجي اما المجموعة الثانية المتمثلة بمجموعة التجربة اعطيت (1ml/kg) المستخلص الكحولي لنبات القيصوم المذاب بواحد مل من الماء فموياً خلال فترة التجربة . وفي نهاية ايام التجربة (ثلاثة اسابيع) قيمت الخصوبة المتمثلة بقياس وزن الجسم وزن الخصى ومعايير السائل المنوي للفئران الاسلاف او المولودة . بالاضافة الى قياس تثخن وقطر انعدام الحيوانات للانابيب التي تم معرفة قياسها من خلال المقاطع النسيجية المنوية التي اخذت لخصى الذكور الاسلاف عند وصولها الى وقت النضج الجنسي .

اظهرت التغيرات المشاهدة في هذا البحث انخفاض معنوي في وزن الجسم , وزن الخصى وكذلك للسائل المنوي لحيوانات التجربة بالمقارنة مع حيوانات السيطرة . بالاضافة الى انخفاض معنوي ($p < 0.05$) في التثخن وقطر انعدام الحيوانات المنوية للأنابيب في حيوانات التجربة مقارنة مع حيوانات السيطرة .