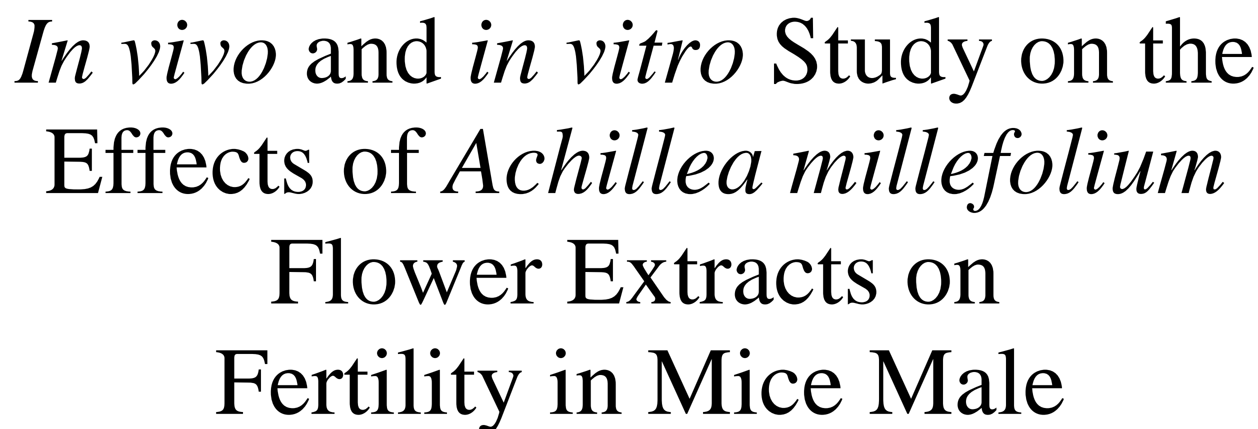

In vivo and *in vitro* Study on the Effects of *Achillea millefolium* Flower Extracts on Fertility in Mice Male

A thesis submitted to the College of Science of AL-Nahrain University
In partial fulfillment of the requirements for the degree of M. Sc. in Biotechnology

By

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الإهداء

إلى من أوجد في نفسي حب العلم ... أبي

إلى من كانت سندي ، بحر الحنان ... أمي

إلى من أحاطني باهتمامه ... أخي

إلى نبعيّ العطاء المستمر ... مشرفيّ

الدكتورة خلود وهيب السامرائي

والاستاذ الدكتور محمد عبد القادر ابراهيم

اهدي ثمرة جهدي المتواضع لهم جميعا عرفانا مني لحسن صنيعهم.

ضفاف

٢٠٠٤

Acknowledgment

I would like to express my sincere appreciation and thanks to my supervisors **Dr. Khulood Al-Sameraei** and **Prof. Dr. Mohammed A. Ibrahim** for their continuous encouragement skilful guidance and patience throughout the research.

Special thanks to the Deanery of the College of Science and to the head of department of Biotechnology / AL-Nahrain University for their help and facilities.

I would like to acknowledge the help and support of the staff in Biotechnology Research Center, especially Dr. **Hazim AL-Ahmad** for his great help.

A special thanks to Miss Rehab, Miss Asmaa, Mr. Mohammad for their help.

My Faithful thanks to my friends Mrs. Rana, Mrs. Reem and Miss Yasamin for their assistance.

Finally I would like to express my sincere thanks to my parents, my brother and my uncle for their support and patience.

DHEFAF

Summary:

This research was conducted to study the effect of *Achillea millefolium* flower extracts on sperm functions of male albino mice both *in vivo* and *in vitro.*

Two extracts that are the aqueous and the ethanolic were prepared from the flowers of *Achillea millefolium*, and then chemical detection was done to identify the active compounds present in each extract. The detected compounds were alkaloids, glycosides, flavonoids, tannins, resins, coumarins, terpenes and saponins.

In vivo effect of the aqueous and ethanolic extracts included assays on the body weight, testis weight and some sperm functions including: motility, concentration, viability and percentage of morphologically abnormal sperms. These two extracts were administered at a dose of 200mg/kg/day, given intraperitoneally for 20 days.

The results showed a decrease in sperm's motility and concentration, and an increase in the percentage of dead and morphological abnormality of sperm, but there was no change body and testes weight.

In-vitro study was carried out on the effect of aqueous and ethanolic extracts on some sperm functions including sperm motility, viability and morphologically abnormal sperms' percentage both after one minute, and 30 minutes of incubation. Each of these two extracts was added at a concentration of 200µg/ml to the sperm suspension. After one minute, the results showed a decrease in sperm motility and an increase in the percentage of dead and morphologically abnormal sperms. While after 30 minutes of incubation, further decrease in sperm motility, and higher increase in sperms' dead and morphologically abnormal percentage were observed.

For both *in vivo* and *in vitro* studies, there was a significant difference between the aqueous and ethanolic extract in which the aqueous extract was more effective on sperm functions than the ethanolic extract, and so the best is the aqueous extract.

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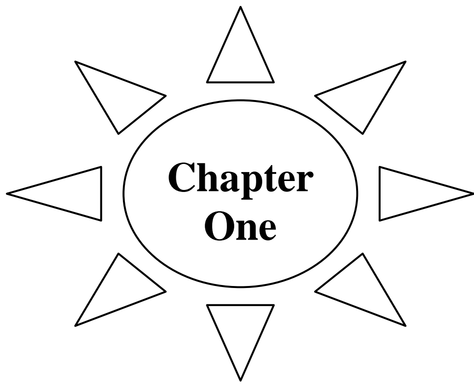
List of Abbreviations

FSH	Follicle Stimulating Hormone
GnRH	Gonadotrophin Releasing Hormone
HCG	Human Chorionic Gondorophin
ISCH	Interstitial Cell-Stimulating Hormone
LH	Leutinizing Hormone



Introduction

and

Literature Review

Introduction and Literature Review

1.1 Introduction:

Herb is a plant or a plant part valued for its medicinal, aromatic or savory qualities. Herb plants produce and contain a variety of chemical substances that acts upon the body. It has been used by all cultures throughout history (Anderson, 1977). Much of the medicinal use of plants seems to have been developed through observations of wild animals by trial and error. Many drugs commonly used today are of herbal origin. The World Health Organization (WHO) notes that of 119 plant-derived pharmaceutical medicines, about 74 % are used in modern medicine in ways that correlated directly with their traditional uses as plant medicines by native people (Barens, 2002).

Herbal medicine is the oldest form of healthcare known to mankind (Anderson, 1977). It sometimes referred to as herbalism or botanical medicine for the use of herbs in therapy or medicinal uses (Barens, 2002). One of the basic targets of herbal medicine is the interaction between different constituents occur, enhancing activity or reducing the adverse effect. Such interaction may be additive or truly synergistic in that compounds interact to produce an effect greater than the sum of individual contribution of each (Williamson, 2001).

Medicinal plants and herbal medicine are one of the current area of investigation in which various drugs have been identified which affect fertility, both in a positive and a negative sense but some of which have side effects that are undesirable (Delaszlo and Henshaw, 1954; Ahmad *et al*., 1998). In spite of considerable development in contraceptive technology, search for male antifertility agents in plants continues to be a potential area of investigation. One method of

finding useful material is the screening of naturally occurring substances to discover those with the optimum characteristics that will guide to the synthesis of the ideal drug (Verma *et al.,* 1980; Choudhary *et al*., 1991). *Achillea millefolium* has been used in popular medicine for its antihemorrhagic and analgesic properties. It was used by north European and North American native people as contraceptive, abortifacient and emmenagogue for women (Chandler *et al*., 1982). Flower extracts of this plant showed histopathological effects on the testes of male mice (Montanari *et al*., 1998).

1.2 The Aims of This Study:

1- To investigate the antifertility activities of active compounds from *Achillea millefolium*.
2- Preparation of extracts from *Achillea millefolium* flowers and detection of some active compounds in these extracts.
3- *In vivo* study on the effect of *Achillea millefolium* flower extracts on body weight, testes weight and some spermatozoa functions in male albino mice.
4- *In vitro* studies on the effect of *Achillea millefolium* flower extracts on some spermatozoa functions in male albino mice.

1.3 Nomenclature:

Scientific name*: Achillea millefolium l.*

Arabic name: Quaisum and Gaisum (Chakravarty, 1976).

English name: Yarrow, milfoil and nosebleed (Hussein, 1985).

Family: Compositae

The name *Achillea* refers to the Greek hero, Achilles, who used the leaves of the plant to check the flow of blood from the wounds of his fellow soldiers in the Trojan War (1200 B. C.), while *millefolium* refers to the featherlike leaves which are so finely divided that the plant appears to have a thousand (Chandler *et al*., 1982). Other names for this plant are: field hops, old man's pepper, sneeze weeds (Hussey, 1974), thousand leaf, thousand weed (Hylton, 1974), bad man's plaything, devil's plaything, herbe militaris (Chandler *et al*., 1982), knight's milfoil and soldier's woundwort (Paulsen, 2002).

1.4 Description:

Achillea millefolium is a perennial herb, generally 30-60 cm tall, it exhibits a characteristic aromatic odor and appears gray-green in color from the numerous small hairs covering it (Chandler *et al*., 1982).

The stem is woolly and tough, alternative leaves are almost green that range from bright-green to grayish and are linear-lanceolate in outline, pinnatly, divided into many small segments, leaflets are sharply cleft and fern-like foilage (Weiner, 1972; Alaa Al-Deen and Micalinico, 1993). The flowers are arranged in dense flat-topped terminal corymbs, white, yellow and pink in color (Chakravarty, 1976; Saad *et al*., 1988). Figure (1-1)

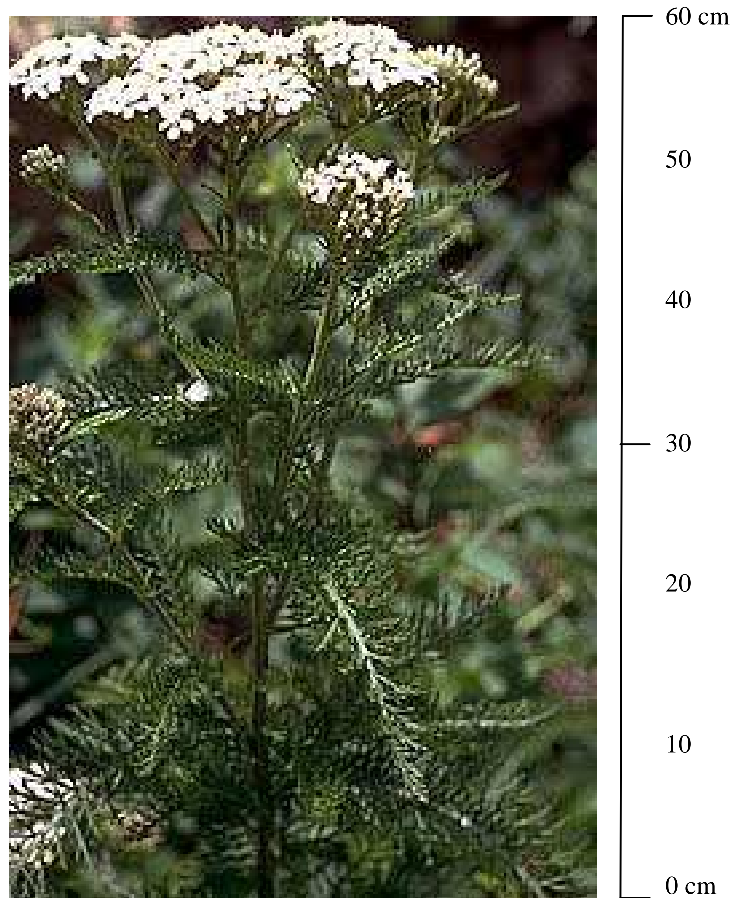


Figure (1-1): ***Achillea millefolium***

1.5 Distribution:

Achillea millefolium grows in open unshaded areas ranging from cliffs and alpine pastures to lowland meadows, roadsides, lawns and waste grounds (Chandler *et al.*, 1982). *Achillea* species are distributed in Asia, Europe, North America and North Africa, especially abundant in the Mediterranean regions, in Egypt, Libya, Lebanon, Palestine and Syria (Ochir *et al.*, 1991; Saad *et al.*, 1988).

In Iraq, it grows in Arbil, Karkuk, Mousl and in the eastern and center of the deposition plane (Majeed and Mahmood, 1988).

1.6 Chemical Components:

Many investigators had studied *Achillea* chemical constituents and had characterized many compounds. The chemical components of *Achillea* can be described as:

1.6.1 Alkaloids:

Achilleine was isolated from *Achillea millefolium* by Zanon and characterized by Miller and Chow (Chandler *et al.*, 1982). Other alkaloids reported to be contained in *Achillea millefolium* were stachydrine, trigonelline (Ziri and Ikram, 1975), achiceine (Saad *et al.*, 1988), and achilletin (Tarify and Mastu, 2000).

Amides and pyrrolideine derivaties were isolated from *Achillea tomentosa* (Harald *et al.*, 1981). Piperideides and pyrrolidides, types of alkaloids were isolated from roots of *Achillea apinulifolia, Achillea grandifolia* and *Achillea lycanica* (Harald *et al.*, 1982). Also 2,3-dehydropiperdide was isolated from *Achillea ptarmica* (Harald and Ferdinand, 1984; Glomibtza, 1987).

1.6.2 Flavonoids:

Flavonoids were first isolated from *Achillea millefolium* by Hoerhammer,

1961. Three flavonoids were detected in the aqueous extract of *Achillea millefolium*, luteolin-7-O-glucoside, apigenin-7-O-glucoside and vitexin (Schulz and Albroscheit, 1988).

Four flavonoids were detected in the flowering tops of *Achillea millefolium*, luteolin, flavonol-3-o-gly., rutin and hyproside (Al-Sawah, 1992).

Other flavonoids reported to be contained in *Achillea millefolium* were artemetin, isorhamentin (Kloshina and Neshta, 1973; Fleming, 1998) and quercetin glycoside (Glasl *et al*., 2002).

Kaempferol was isolated from *Achillea asiatica* (Narantuya *et al*., 1999), while two flavonoids isolated from *Achillea cretica*, were isoorientin and isoorientin-7-O-methyl-ester (Anon, 2001).

1.6.3 Volatile Oils:

The volatile oil of *Achillea millefolium* was first isolated by distillation, which marks the beginning of chemical analysis of this plant (Chandler *et al*., 1982). The investigation of essential oil composition of *Achillea millefolium* had led to the isolation of monoterpene hydrocarbons with α-pinene, α-thujone and β-ocimene (Engel and Tressel, 1983).

The essential oil isolated from the aerial parts of *Achillea millefolium* contained many compounds, the major were found to be β -thujone, camphor, 1,8-cineole, β-pinene and sabinene (Jean-Marie *et al*., 1990).

Monoterpene alcohols were isolated from *Achillea millefolium*, the major components were linalool, α-terponeol, manthane and borneol (Ludwing *et al*., 1992).

Another investigation had led to the isolation of essential oil from the Hungarian *Achillea millefolium* contained significant amounts of chamazulene (Verzar-Petri and Shalaby, 1977; Shalaby and Verzar-Petri, 1979; Alaa AL-Deen and Micalinico, 1993).

Other components isolated from *Achillea millefolium* volatile oil were bornyl acetate (Chelishvili and Tavberidze, 1974), eugenol, furfural, humulene (Falk *et al*., 1974) and caryophyllene (AL-Shahat, 1992).

The volatile oil isolated from the flowering tops of *Achillea fragrantissima* contained α-thujone, β-thujone and 1,8-cineole (Abutabl *et al*., 1986).

Four terpenic volatile oils isolated from *Achillea asiatica* were α-pinene and β- pinene, camphene and α-limonine, while bornyl acetate and 4-terpineols were isolated from the essential oil of *Achillea herba-rota*, also seven terpenic volatile oils, camphor, bornyl acetate, 4-terpineol, neryl acetate, bornyl, geraniol and achillinol acetate were isolated from *Achillea grandiflora* (Al-Shahat, 1992).

The essential oil isolated from *Achillea lycaonica* contained sabinene, while borneol and terpinen-4-ol were the major constituents isolated from *Achillea ketenoglui* (Basar *et al*., 2001).

Three essential oils including 1,8-cineole, camphene and camphor were isolated from *Achillea cithemifolia* (Florian *et al*., 2002).

Unlu *et al*., 2002 isolated the essential oil of *Achillea setacea* and *Achillea terefolia* air-dried parts, the identified compounds were 1,8-cineole, camphor and borneol.

1.6.4 Triterpenes and Sterols:

The four triterpenes isolated from *Achillea millefolium* aireal parts, were α-amyrin which is the major triterpene, β-amyrin, taraxasterol and pseudotarasterol (Chandler *et al*., 1982). β-sitosterol was isolated from *Achillea schischkini* (Ulubelen *et al*., 1989), α-amyrin and stigmasterol were isolated from *Achillea sintenissi* of central Turkey (Neuzhun *et al*., 1988).

A new triterpene called magnificol, α-amyrin and the sterolic compound called β- sitosterol were isolated from *Achillea magnifica* aireal parts (Ulubelen *et al*., 1989).

Four sterols were isolated from *Achillea millefolium*, β-sitosterol was the major sterol, stigmasterol, campesterol and cholesterol (Glasl *et al*., 2002).

1.6.5 Sesquiterpene Lactones:

Three sesquiterpene lactones isolated from *Achillea millefolium* were, achillin, 8-anelooxy-artabsin and 2,3- dihydrodesactoxy matricin (Verzar- Petri *et al*., 1977).

Banh-NHV *et al*., 1979 had isolated achillicin the major proazulene of sesquiterpere lactones of *Achilla millefolium*.

Two investigations in *Achillea millefolium* had led to the isolation of α-peroxyachifolid which is the most sesquiterpene lactone allergin (Hauson *et al*., 1991; Paulsen *et al*., 1993; Rucker *et al*., 1994; Paulsen *et al*., 2001).

Leucodin and desacetyl matricarin were isolated from Hungarian *Achillea millefolium* (Glasl *et al*., 2003).

Other sesquiterpene lactones isolated from *Achillea millefolium* were: tricyclene, α-terpinene (Falk *et al*., 1974), millefin and milletin (Seaman, 1982).

A new sesquiterpene lactone, sintene was isolated from *Achillea sintanissi* aireal parts of Turkey (Nezhun *et al*., 1988).

Three main prozulenes, 8α-angeloxy-artabsin, 8α-tigloxy-artabsin and achillicin, and four guaianolides were isolated from Mangolian *Achillea asiatica* aireal parts (Ludwig *et al*., 1992; Glasl *et al*., 2001).

Many investigations showed that guaianolide type is dominant in sesquiterpene lactone of *Achillea roseoalba* (Kastner *et al*., 1991), *Achillea asplenifolia* (Kastner *et al*., 1992 and Schroder *et al*., 1994), *Achillea ceretanica* (Glasl *et al*., 1997; 1998) and *Achillea setacea* (Kubelka *et al*., 1999).

Endesmarolide was isolated from *Achillea pratensis* (Saukel and langer, 1992), longiopinene from *Achillea distans* (Saukel, 1994), germancrare from *Achillea pannouica* (Wurzinger, 1995), and matricarin and achillicin from *Achillea*

collina (Glasl *et al*., 1999).

Other active constituents of this plant are resins (Foster and Duke, 1990), saponins (Wichtl, 1994), tannins and coumarins (Freeman, 2001).

1.7 Non Medical Uses:

Beside its many medical applications, some other interesting and useful attributes of *Achillea millefolium* have been reported. *Achillea millefolium* has been used as food flavoring, additive to salad, poultry dishes and baked Spanish recipes (Hylton, 1974). In Sweden it is used instead of hops in the manufacture of beer (Millspaugh, 1974). Moreover other investigators reported that the ethanolic extract and the burned dried *Achillea millefolium,* and the aqueous extract of *Achillea santolina* aerial parts were used to repel mosquitoes and flies (Lalonde *et al*., 1980; Abdul-Amir, 1981).

The aqueous extract of *Achillea santolina* aerial parts had effect against *Tribolium confusum, Oryzaephiluls surinamensis* and *Trogoderma granarium* insects (Abdul- Amir, 1981).

The oil of *Achillea millefolium* has traditionally used as a hair shampoo (Grieve, 1974) and in the cosmetic application (Anon, 2001).

1.8 Medical Uses:

Achillea millefolium was used to treat ulcer, earache, pneumonia, small pox (Levy, 1966), tuberculosis (Korchmal and Korchmal, 1973) and swollen tissue (Hussey, 1974).

Krochmal and Korchmal, 1973 mentioned that *Achillea millefolium* tannins have astringent activity while resins have a diuretic activity.

The Greek hero, Achilles, used the leaves of *Achillea millefolium* to check the flow of blood from the wounds of his fellow soldiers (Chandler *et al*., 1982), so the

traditional herbalists in Europe, China and India used this herb to stop minor bleeding and to treat minor wounds (Kastner *et al*., 1993).

Chewing of *Achillea millefolium* leaves was used by north American Indians to relieve toothache pain (Chandler *et al*., 1982).

In Iraq, the aqueous extract of *Achillea millefolium* has been used to stop hemmorrhoidal bleeding as hemostatic (Saad *et al*., 1988).

The aqueous extract made from the whole plant is used as a lotion to treat sore eyes, mixing of this herb with cold water has been used to treat burns (Jonston, 1970), while the aqueous extract of *Achillea millefolium* flower heads has anti-inflammatory activity in a number of conditions, especially in the intestinal and female reproductive tract as mild sedative (Haggog *et al*., 1975; Reynolds, 1993).

Three sesquiterpenes of *Achillea millefolium*, achimillic acid A, B and C had a strong activity against abnormal blood cells in live mice (Foster and Duke, 1990).

Tea made from leaves and flower of *Achillea millefolium* was used for stomach trouble (Johnston, 1970), common cold / sore throat, indigestion, and as hypotensive (Al-Shahat, 1992).

Al- Shahat, 1992 mentioned that the hemostatic activity of *Achillea millefolium* was referred to the two alkaloids, achilleine and achilletin which can stop the flow of blood from wounds.

Achillea millefolium was known as appetite stimulant for treating anorexia and though its bitter properties, it increase the flow of gastric juice relieving dyspepsia and abdominal cramps (Jurenitsch, 1992).

A study on sesquiterpenes isolated from *Achillea millefolium* showed antitumor activity against leukemia cells of mice (Tozyo *et al*., 1994).

The oil of *Achillea millefolium* was used in folk medicine in a form of ointment to treat varicose veins, skin problems, as a local anesthetic to relieve pain (Haussen and Vieluf, 1997), and has antimicrobial activity against *Acinetobacter lowffi, Clostridium perfringens, Mycobacterium smegmatis, Staphylococcus*

pneumoniae, Candida albicans and Candida krusei (Unlu *et al*., 2003).

Achillea millefolium is an old medicinal plant used by north European and North American primitive people as contraceptive (Delaszlo and Henshaw, 1954), abortifacient (Angier, 1974; Millspaugh, 1974; Albert-Puleo, 1978) and emmenagogue (Hussey, 1974; Jaber, 1991).

Achillea santolina was used in folk medicine as carminative, tonic, to treat abdominal cramps and dysentery (Chakravatry *et al*., 1976), in addition to its anti-inflammatory activity when tested on rats (Al- Hindawi *et al*., 1988).

The essential oil isolated from *Achillea fragrantissima* has carminative, anthelmentic activity (Sinkary, 1977), and showed a marked antimicrobial activity against *E. coli*, *Bacillus subtilis* and *Staphylococcus aureus* (Barel *et al*., 1991).

In Chinese folk medicine *Achillea sibirica* was used to relieve headache (Chandler *et al*., 1982).

Flavonoids of *Achillea millefolium* have antispasmodic activity (Haussen and Vieluf, 1997).

Flavones isolated from the aerial parts of *Achillea atrata* showed antimicrobial activity against *Bacillus subtilis* and *Candida albicans* (Aljancic, *et al*., 1999).

The ethanolic extract of *Achillea millefolium* has anti-hyperglycemic effect in mice (Petlevski *et al*., 2001) and a moderate antibacterial activity against *Staphylococcus aureus, E.coli, Bacillus subtilus, Mycobacterium smegmatis, Shigella sonnei and Shigella flexnii* (Freeman, 2001).

1.9 The Reproductive System of Male Mouse:

The reproductive system of male mouse consist of the primary reproductive organs, the testes and the secondary reproductive organs, which include the scrotum, epididymis, ductus deferens, seminal vesicles, coagulating glands, urethra, prostate glands, bublourethral glands (Seeley *et al.,* 1996) as in figure (1-2).

The testis is a paired oval tubular gland in which the male sex cells, the spermatozoa, develop. They are located in scrotal sacs which lies just anterior to the anus, on either side of the urethra (Foster *et al*., 1982).

This organ is covered by a fibrous connective tissue capsule, the tunica albuginea, from which at the hilus, thin septa projects into the gland and divide it into lobules.

These lobules contain convoluted seminiferous tubules (Bearden and Faquay, 1992; Saladin and Porth, 1998). The walls of these tubules are lined with two types of cells: spermatogenic cells, which give rise to sperm and Sertoli cells.

Between the seminiferous tubules are clusters of endocrine cells, called interstitial endocrinocytes or leyding cells, which secrete the male sex hormone, testosterone (Mader, 1993; Miller and Harley, 1996).

The seminiferous tubules empty into tubular network called the rete testis. The rete testis empties into three to five tubules called efferent ductules, these efferent ductules exit the testis into the epididymis. The epididymis is a tightly coiled series of thread like tubule located at the posterior side of the testis and it consists of three parts, the caput epididymis (head), corpus epididymis (body) and the cauda epididymis (tail) (Foster *et al*., 1982). The epididymal functions include transport, as a duct system leading from the testis to the ductus deferens, concentration, storage of spermatozoa until they mature and ready to be ejaculated, maturation in which they acquire motility and fertilizing capacity during passage through the

epididymis and loss of cytoplasmic droplet which formed during spermatogenesis

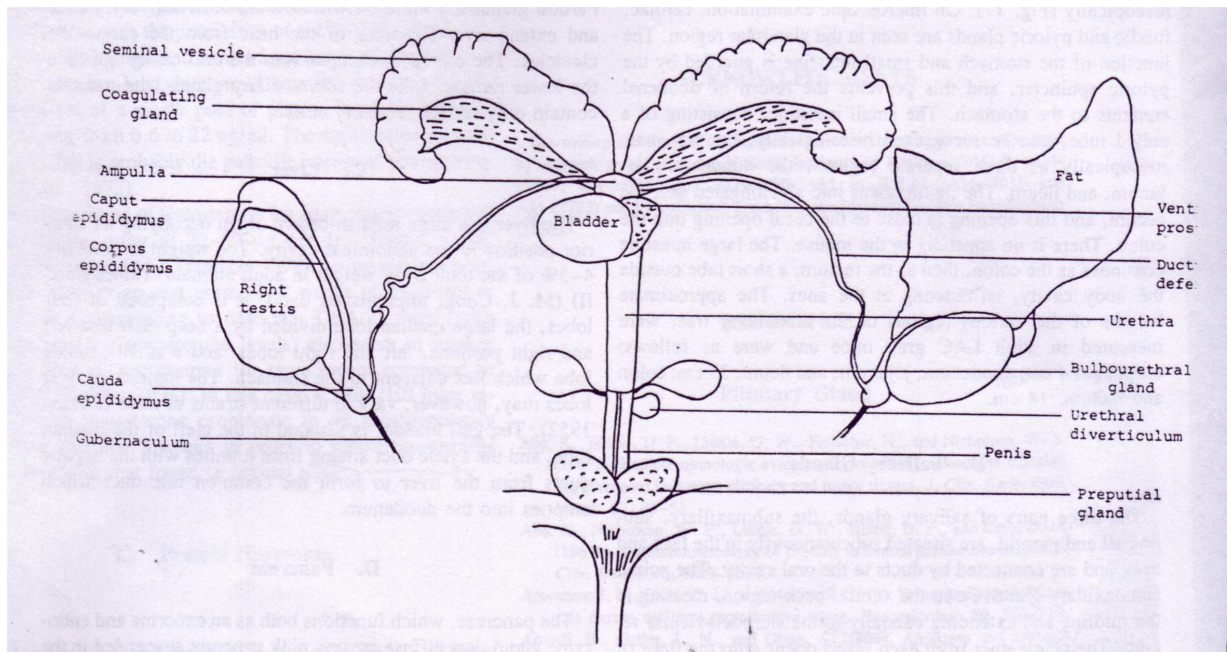


Figure (1-2): Reproductive system of male mouse (Foster *et al.,* 1982).

(Austin and Short, 1982; Knobit and Neill, 1998; Martini and Mark, 1998). The ductus deferens emerges from the cauda epididymis and widens into an ampulla before entering the dorsal wall of the urethra near the neck of the bladder.

The paired seminal vesicles are white, curved, elongated structures notched on the convex surface and hooked at the lateral tips. Each gland has a wide duct which enters the urethra with the ampulla of the ductus deferens. The paired coagulating glands are less conspicuous than seminal vesicles and are translucent in appearance. The paired ventral prostate glands are pinkish in color having several ducts which empty into the urethra on the ventral wall. A third pair of prostate glands which lie dorsal to the urethra open laterally into the urethra. The paired bulbouretral (Cowpers) gland lie lateral to the junction of the membranous urethra and penis (Foster *et al*., 1982).

1.9.1 Spermatogenesis:

The entire process of sperm formation, beginning with spermatogonia and resulting in mature spermatozoa is referred to as spermatogenesis, this process occurs in the seminiferous tubules of the testis. The seminiferous epithelium lining the seminiferous tubule is composed of two basic cell types: the developing germ cells and the Sertoli cells (Guyton, 1989; Kalthoff, 2001).

The germ cells undergo a continuous series of cellular division and developmental changes, beginning at the periphery and progressing toward the lumen of the tubule in which each stage is little closer to the lumen of the tubule than the earlier stage (Saladin and Porth, 1998; Hafez and Hafez, 2000).

The stem cells, called spermatogonia, divide through mitosis in which some daughter cells produced from these mitotic divisions remain as spermatogonia and continue to divide by mitosis. Other daughter cells form primary spermatocytes.

These primary spermatocytes undergo meiosis I giving rise to haploid secondary spermatocytes and each of these undergoes meiosis II to produce spermatids; each stage is a little closer to the lumen of the tubule than the earlier stage (Dorit *et al.*, 1991; Seeley *et al.*, 1996; Kalthoff, 2001), figure (1-3), the spermatids are then transformed into spermatozoa by a series of morphologic changes collectively known as spermiogenesis. These changes include condensation of the nuclear chromatin, formation of the sperm tail, and development of the acrosomal cap (Arab *et al.*, 1989; Austin and Short, 1982; Hafez and Hafez, 2000). The fully formed spermatozoa are extruded into the lumen of the seminiferous tubules by a process called spermiation (Berne and Levy, 1988).

1.9.2 Hormonal Control of Male Reproduction:

Hormonal mechanisms that influence the male reproductive system involve the hypothalamus of the brain, the anterior pituitary gland and the testis (Sieger, 1997).

Gonadotropin-releasing hormone (GnRH) is released from the hypothalamus which stimulates the anterior pituitary gland to secrete luteinizing hormone (LH) and follicle stimulating hormone (FSH) in the blood.

LH stimulates the leyding cells to secret testosterone, it is sometimes given the name interstitial cell-stimulating hormone (ICSH) because it controls the production of testosterone by the interstitial cells which are scattered in the spaces between the seminiferous tubules. FSH stimulates the sertoli cells in the seminiferous tubules to facilitate sperm development and to secret inhibin and activin, which regulates the FSH secretion (Ijam *et al.*, 1990; Mader, 1993).

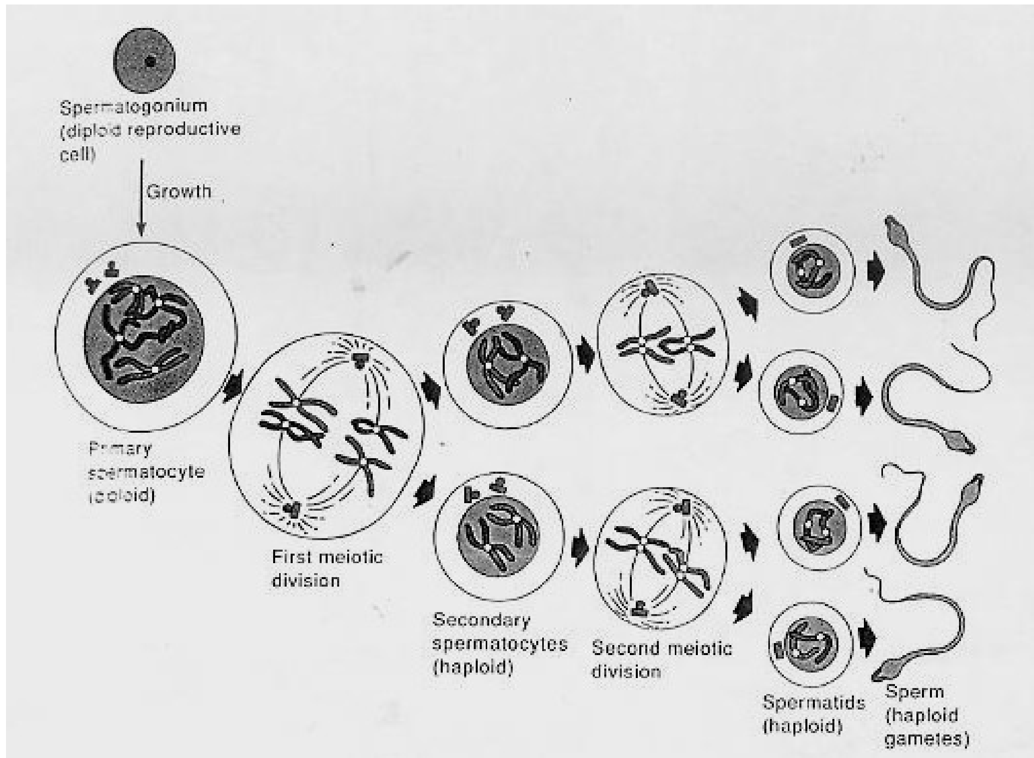


Figure (1-3): Spermatogenesis (Miller and Harley, 1996).

Testosterone is necessary for proper sperm production, development and maintenance of male reproductive organs and stimulates development of male secondary sexual characteristics. Regulation of hormonal status is achieved throughout feedback mechanism. Both inhibin and activin acting as feedback regulators of FSH release (Lin *et al*., 1989; Vliegen *et al.,* 1993). Inhibin exerts inhibitory effect (Ying, 1988), where as activin stimulates FSH secretion (Lin *et al*., 1989). Figure (1-4).

Testosterone has a negative-feedback effect of the hypothalamus and pituitary gland to reduce LH and FSH secretion (Raven and Johanson, 1996; Seeley *et al*., 1996).

1.9.3 The Spermatozoon Morphology and Structure:

The spermatozoon consists of a head and a tail, like other cells the spermatozoon is enclosed within the plasma membrane.

The shape of the sperm head is characteristics of the species, in mouse it is hook-shaped, and it is composed of two parts, the nucleus and the acrosome. The nucleus contains a highly condensed chromatin, the acrosome is surrounded by the acrosomal membranes and covers the anterior part of the sperm nucleus, it contains enzymes, important in pentration of the egg in the fertilization process (Austin and Short; 1972,1982; Sieger, 1997; Saladin and Porth, 1998). The tail is divided into a neck middle piece, principle piece and the end piece. The neck connects the tail to the head of spermatozoon.

The middle piece is composed of the axial filaments or axoneme (nine pairs of microtubules arranged around two central microtubules) surrounded by nine coarse fibers which is in turn surrounded by the mitochondrial sheath. The principle piece consists of the axoneme surrounded by a fibrous sheath, while the end piece is composed of the axoneme only enclosed within the plasma membrane (Austin and

Short, 1972; Martini and Barholomew, 1999; Hafez and Hafez, 2000).

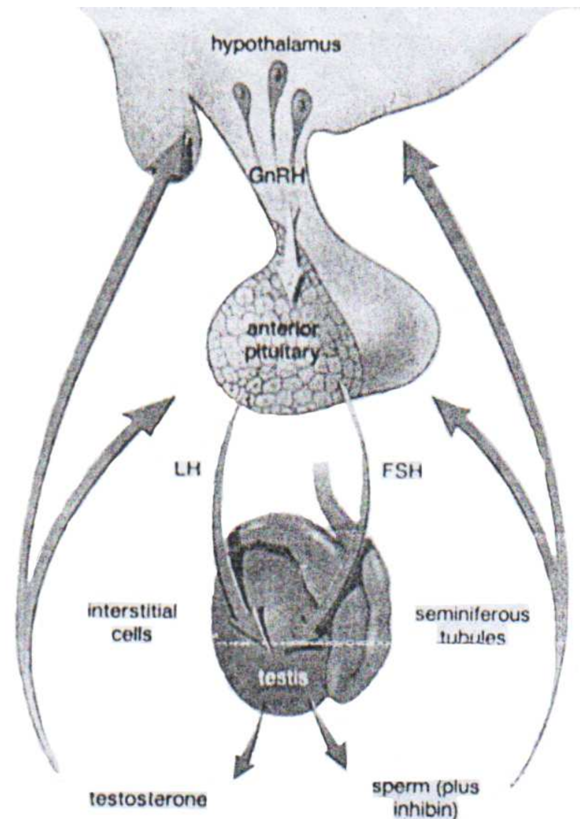


Figure (1-4) Regulation of reproductive hormone secretion in male (Mader, 1993)

1-9-4 Contraception

Contraception is the prevention of conception or impregnation. Many methods are used to prevent the release and transport of both male and female gametes (Sood, 1994). Prevention or termination of pregnancy including methods that prevent fertilization, prevent implantation of the developing embryo and removing the implanted embryo or fetus (abortion) (Seeley *et al.*, 1996). However, contraceptives are most common used drugs, could be obtained from two sources, chemical synthesis and from plants.

Many experiments had proved that the effect of synthetic chemical compounds do not give the same physiological activity as the active compounds from medicinal plants do. Moreover, synthetic chemical substances have been shown to have many adverse side effects (Hussein, 1981).

Several investigators classified various contraceptives according to their effects on male and female:

1. Male contraception, which include:

A- Sterilization (surgical method): it is a surgical removal of a portion of the tubes through which the gametes are transported from the gonads, in male (vasectomy) (Raven and Johnson, 1996).

B- Behavioral method: it includes abstinence and coitus interrupts.

C- Barrier method: it includes condoms and spermicidal gels (Seeley *et al.*, 1996).

2. Female:

There are many methods used used:

A- Sterilization (Surgical method): it includes tubal ligation hysterectomy (Raven and Johnson, 1996).

B- Barrier method: including vaginal condom, diaphram, cervical cap,

spermicidal creams, jellies and foams, spermicidal douches and lactation.

C- Prevention of ovulation: oral contraceptives (birth control pills) are used.

D- Prevention of implantation: intrauterine devices (IUDs) are used.

E- Abortive methods: it includes curettage and RU-486.

F- Other methods: including injectible contraceptive and implants (Norplant) (Raven and Johnson, 1996).

Many investigators have shown the effects of some plants on fertility in females. Oral administration of *Juniperus communis* fruits at a dose of 500 mg / Kg resulted in 100% anti-implantation in female albino rats (Agrawal *et al*., 1980).

The fruit extract of *Cassia fistula* linn. has anti-implantation effect in female albino rats (Bhardwaj *et al*., 1980).

The dried berriers of an Indian herb, *Embelia ribes* has anti-implantation activity in rates of 50 mg / Kg / day for 7 days and antiestrogenic activity (Parkash, 1981). Zoapatle is a decoction made from *Montanoa tomentosa* leaves, which has been used as an oral contraceptive in traditional Mexican medicine (Gallegos, 1983).

Seeds of *Sesbania sesban* had showed 100% antifertility activity in female rats at 250 and 400 mg / Kg doses on the 10th day of pregnancy (Singh, 1990).

Gossypol isolated from *Gossypium hirsutum* acts as antifertility in female by disrupting oestrus cycle, pregnancy and early embryo development in nonruminant animals (Randel *et al*., 1992).

The methanolic extract of *Asparagus pubescens* roots at a dose of 0.5-1.5 g / Kg protected mice, rats and rabbits from conception and inhibits fetal implantation (Nwafor *et al*., 1998).

Oral administration of *Pergularia daemia* ethanolic extract at 200, 400 and 600 mg / Kg body weight daily resulted in pregnancy termination at the preimplantation stage (Sadik *et al*., 2001), while the alkaloidal fraction of this extract of the same plant at a dose of 200 mg / Kg body weight showed a

significant activity in preimplantation stage of female mice (Sadik *et al*., 2001).

Gupta *et al*., 2002 had reported that *Marsila minuta* methanolic extract had antifertility activity in female Swiss albino mice.

The alcoholic extract of *Thespesia populrea* seeds at a dose of 110 mg / Kg had 60% inhibition of implantation while both doses of 120 and 150 mg / Kg had 100% inhibition of implantation when taken orally by female rats from first day to seventh day of pregnancy (Ghosh and Bhattachyarya, 2004).

1.9.5 Effect of Some Plants on Sperms:

Various investigators had studied the effect of various plants extract on sperms:

Treatment of mice with 3 g / Kg / day of *Artemisia abyssinica* ethanolic extract for three months caused a decrease in both sperm concentration and motility (Qureshi, *et al*., 1990).

Intraperitoneal administration of *Hibiscus rosa sinesis* chloroform and alcoholic extracts at a dose of 125 and 250 mg / Kg / day for 20 day into male albino mice resulted in a decrease of epididymal sperm concentration (Reddy *et al*., 1995).

Oral administration of 0.4 g / 100 g body weight of *Hypericium crispum* leaves for 10, 20, 30 and 40 days into male mice caused a low sperm concentration and an increase in the percentage of dead and morphologically abnormal sperms (Khalil, 1998).

Administration of 50 µg / Kg/ day of Tripchloride isolated from *Triptergium wilfordii* orally for 5 weeks had a decreasing effect on cauda epididymal sperm density and motility with an increase in the percentage of abnormal sperms in male rats (Wang *et al*., 1999).

Aladakatti *et al*., (2001) had reported that treatment of male albino rats with *Azaderachta indica* leaves caused a decrease in total sperm count, motililty and an

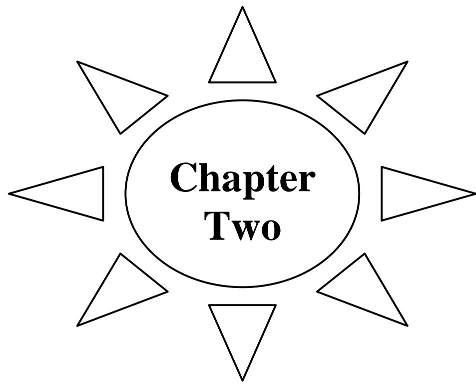
increase in the percentage of abnormal sperms.

Treatment of male rats with 50, 100 and 200 mg / Kg / day for 60 day by oral administration of *Martynia annua* root ethanolic extract caused a reduction in epididymal sperm count and motility (Mali *et al*., 2002).

In male albino mice, the petroleum ether extract at the dose of 10 and 20 mg / mouse per day for 20, 40 and 60 day of *Mentha arvensis* leaves, when administered orally, showed a decrease of caudal epididymis sperm count, motility, viability and normal morphology (Sharma and Jacob, 2001; Sharma and Jacob,2002).

The oral administration of *Amalakyadi churna* ethanolic extract at a dose of 250 and 400 mg / Kg / day for 30 days in male mice caused a decrease in sperm count (Seetharma, 2003).

An investigation on the effect of treatment with 500 mg / Kg / day for 7 days of *Curcuma comosa* hexane extract oral administration in adult male rats resulted in a decrease of epididymal sperm concentration and motility (Piyachaturawat *et al*., 2003).



Materials and Methods

Materials and Methods

2.1 Plant Collection:

Achillea millefolium was collected in 2003 between May and July at morning from the roadsides leading between Baghdad to Mousl.

Aerial parts of this plant were air dried in shade at room temperature, then the flowers were separated and grinded into powder form by using electric grinder.

2.2 Animals:

Healthy adult male albino mice were obtained from the animal house of Biotechnology Research Center/Al- Nahrain University.

Forty male mice were used in this study, the age of these mice was in the range between 10-12 week old, and their weight was around 28-38 gram. These animals were kept under suitable environmental conditions of 20-25°C and photoperiod of 12 hour daily

2.3 Preparation of Reagents, Solutions, Media and Stain for the Study:

2.3.1 Reagents:

The following reagents are used for the detection of some active compounds in the plant extracts:

2.3.1.1 Wagner's Reagent:

Two grams of potassium iodide were dissolved in 5ml of distilled water, then 1.27g of iodine was added and stirred until dissolved, the volume was completed to 100ml by adding distilled water (Smolenski *et al.*, 1972). This reagent is used for

the detection of alkaloids.

2.3.1.2 Mayer's Reagent:

Solution A: 358 g of mercuric chloride were dissolved in 60 ml of distilled water.

Solution B: 5 g of potassium iodide were dissolved in 10 ml of distilled water A and B solutions were mixed, the volume was completed to 100 ml by adding distilled water (Smolenski *et al.*, 1972).

This reagent was used for the detection of alkaloids.

2.3.1.3 Dragendorff's Reagent:

Two stock solutions were prepared:

Solution A: 0.6 g of bismuth subnitrate was dissolved in 2 ml of concentrated hydrochloric acid and 10 ml of distilled water.

Solution B: 6 g of potassium iodide were dissolved in 10 ml of distilled water. Solution A and B were mixed together with 7 ml of concentrated hydrochloric acid and 15 ml of distilled water, and the whole was completed to 400 ml by adding distilled water (Harborne, 1973).

This reagent was used for the detection of alkaloids.

2.3.1.4 Fehling Reagent:

Solution A: 35 g of copper sulfate were dissolved 100 ml of distilled water, then diluted by distilled water till volume 500 ml.

Solution B: 7 g of sodium hydroxide and 175 g of roshail salt were dissolved in 100 ml of distilled water, the volume was completed till 500 ml by adding distilled water.

A and B solutions were mixed in equal volumes (Sarkas *et al.*, 1980).

This reagent was used for glycosides detection.

2.3.2 Solutions:

The following first three solutions were used for the detection of some active compounds in the plant extracts:

2.3.2.1 Lead Acetate Solution 1% (w/v):

Prepared by dissolving 1 g of lead acetate in 100 ml distilled water, it's used for tannins detection.

2.3.2.2 Ferric Chloride Solution 1% (w/v):

Prepared by dissolving 1 g of ferric chloride in 100 ml distilled water, it's used for tannins detection.

2.3.2.3 Potassium Hydroxide Solution 50% (w/v):

Prepared by dissolving 50 g of potassium hydroxide in 100 ml of distilled water. This solution is used for the detection of flavonoids.

2.3.2.4 Normal Saline Solution (0.9%):

Prepared by dissolving 0.9 g of sodium chloride in 100 ml of distilled water. This solution was used in various *in vivo* assays including dilution of the plant extract and for the control group injection.

2.3.2.5 Diluting Solution:

This solution composed of 1 ml of 2 % eosin, 1 ml of 3 % sodium chloride and 50 ml of distilled water (Zaid and Salhab, 1994); it is used for determination of sperm concentration.

2.3.3 Media:

Roswell Park Memorial Institute-1640 (RPMI-1640) medium was used with the additives showed in table (2-1) to prepare the sperm suspension.

Table (2-1) Media used for sperm suspension.

Content	Amount
RPMI-1640	90%
Fetal calf serum (FCS)	10%
FSH	1 mg / 100 ml
HCG	1 mg / 100 ml
β-estradiol	100 μg / 100 ml
Penicillin	100 I.U / ml
Streptomycin	10 mg / 100 ml
Sodium pyruvate	11.52 mg / 100 ml
pH	7.4

The media was sterilized by filtration through millipore filter (0.22 um) and stored at 4 °C (Al-Deraji, 2002).

2.3.4 Stain:

2.3.4.1 Eosin-Nigrosin Stain:

It is prepared by dissolving 1g eosin, 2 g nigrosin and 2.9 g sodium citrate in 100 ml distilled water (Bearden and Faquay, 1992). This stain was used for the assessment of dead and live sperms in which dead sperm takes up the red stain while live sperm does not, as well as for determination of morphological abnormality percentage of sperms.

2.4 Preparation of Plant Extracts:

2.4.1 Aqueous Extract:

The flowers powder of *Achillea millefolium* was macerated with sterilized distilled water in ratio of 1:7 (50 g of the powder in 350 ml of sterilized distilled water). During extraction the mixture was shaked continuously for 6-7 hours at room temperature in a shaking incubator, the suspension was filtered and the filtrate was concentrated using vacuum rotary evaporator at 40 ˚C.

2.4.2 Ethanolic Extract:

Fifty grams of *Achillea millefolium* flowers powder were extracted with 500 ml of 96% ethanol under continuous stirring for few hours at room temperature, the suspension was filtered and the filtrate was concentrated using vacuum rotary evaporator at 40 ˚C (Montanari *et al.*, 1998).

2.5 Measurement of the Extract Acidity:

Ten grams of the flower powder were mixed with 50ml of distilled water for 10

minutes by magnetic stirrer, then the suspension was filtered and the acidity of the filtrate was measured using pH meter (Shihata, 1951). Also the acidity of the ethanolic extract was measured.

2.6 Detection of Some Active Compounds in *Achillea millefolium* Flower Extracts:

2.6.1 Detection of Alkaloids:

10 ml of the concentrated plant extract acidified by 4% of 13N hydrochloric acid were tested with the following reagents to ascertain the presence of alkaloids:

1-Wagner's reagent: appearance of brown precipitate is an indication for the presence of alkaloids (Hussein, 1981).

2-Mayer's reagent: appearance of white precipitate is an indication for the presence of alkaloids (Hussein, 1981).

3-Drangendorff's reagent: appearance of orange precipitate is an indication for the presence of alkaloids (Fahmy, 1933).

2.6.2 Detection of Glycosides:

-Non-hydrolyzed extract:

Equal volumes of the plant extract and fehling reagent were mixed, then left in a boiling water bath for 10 minutes, the appearance red precipitate indicates the presence of glycosides.

- Hydrolyzed extract:

Few drops of 10 % hydrochloric acid were added to 5 ml of the plant extract, then left in a boiling water bath for 20 minutes, the acidity was neutralized by adding few drops of sodium hydroxide solution, equal volume of fehling reagent was added, the appearance of red precipitate is an indication for the aglycon part of the glycoside (Shihata, 1951).

2.6.3 Detection of Flavonoids:

Ten ml of 50 % ethyl alcohol were mixed with 10 ml of 50 % potassium hydroxide solution, then added to equal volume of the plant extract, the appearance of yellow color indicates the presence of flavonoids (Jaffer, 1983).

2.6.4 Detection of Tannins:

Ten ml of the plant extract was divided into two equal parts:

1- Few drops of 1 % lead acetate solution were added to part one, the appearance of gelatinous white precipitate indicates the presence of tannins.

2-Few drops of 1% ferric chloride solution were added to part two; the appearance of greenish-blue color indicates the presence of tannins (Shihata, 1951).

2.6.5 Detection of Saponins:

i. Plant extract was shacked vigorously in a test tube, the formation of foam remaining for few minutes indicated the presence of saponins.

ii. Five milliliters of the plant extract were added to 3 ml of mercuric chloride solution, the appearance of white precipitate indicated the presence of saponins (Shihata, 1951).

2.6.6 Detection of Resins:

10 ml of distilled water acidified with 4% hydrochloric acid were added to 5 ml of the plant extract, the appearance of turbidity indicated the presence of resins (Shihata, 1951).

2.6.7 Detection of Terpenes:

One gram of the plant extract was precipitated in a few drops of chloroform, and then a drop of acetic anhydride and a drop of concentrated sulfuric acid were added, the appearance of brown color represents the presence of terpenes (Al-Abid, 1985).

2.6.8 Detection of Coumarins:

Five milliliters of the plant extract were put in a test tube, then covered by a wet filter paper with 1N sodium hydroxide solution, the test tube was placed in a boiling water bath for 10 minutes, after that the filter paper was exposed to UV-light source, the appearance of greenish-yellow color indicates the presence of coumarins (Giesman, 1962).

2.7 Collection and Preparation of Sperms:

The sperms were collected from the epididymis of mice by the following steps:

1- Mice were killed by cervical dislocation and dissected directly.

2- The caudal epididymis was cut and placed in a petridish containing 0.9 ml of RPMI-1640 medium and minced by using microsurgical scissor and forceps (Hassan *et al*., 1983; Zhao *et al*., 2001).

2.8 *In vivo* and *in vitro* Treatment Assays:

2.8.1 *In vivo* Treatment:

In this experiment five mice were used per each of the following treatments:

A- Control A (untreated).

B- Control B (distilled water treated).

C- Control C (tween-20 and saline treated).

D- Aqueous extract treated.

E- Ethanolic extract treated.

2.8.1.1 Aqueous Extract Treatment:

The aqueous extract was administered at a dose of 200mg/kg/day, intraperitoneally in mice for 20 days. The control group B of this treatment included mice were injected with the same volume of distilled water.

2.8.1.2 Ethanolic Extract Treatment:

The ethanolic extract was suspended in 7 ml of tween-20 and diluted by saline solution, then administered at a dose of 200 mg/Kg/day, intraperitoneally in mice for 20 days. The control group C of this treatment included mice were injected with the same volume of tween-20 and saline solution.

2.8.2 *In vitro* Treatment:

Five mice were used in each of these experiments:

A- Control (untreated).

B- Aqueous extract treated.

C- Ethanolic extract treated.

2.8.2.1 Aqueous Extract:

The aqueous extract was added at a concentration of 200µg/ml of the sperm suspension. The measurements were carried out after one minute, and 30 minutes after the addition of the plant extract.

2.8.2.2 Ethanolic Extract:

The ethanolic extract was added at a concentration of 200µg/ml to the sperm suspension. The tests were done after one minute, and 30 minutes after the addition of the plant extract.

2.8.2.3 Control Group:

The sperm suspension prepared previously in (2-8) was tested at time 0 and 30 minutes without any treatment.

2.9 Tests for the *in vivo* Study:

2.9.1 Body Weight:

Mice were weighed at the beginning of the experiment, at weekly intervals and at the end of the experiment, the weight was expressed in gram (g) (Montanari *et al*., 1998).

2.9.2 Testes Weight:

Both testes of each mouse were weighed and expressed in terms of mg/100 g body weight.

2.9.3 Sperm Functions:

2.9.3.1 Sperm Motility:

Sperm motility was assessed according to the method reported by Bearden and Faquay, 1992. The method was as follows:

i. 50 µl of the sperm suspension prepared in (2.8) was placed over a slide and covered by a cover slide.
ii. Using light microscope, several fields were examined to estimate the percentage of individual motility of sperms.
iii. The percentage of individual motility was assessed according to the method reported by Ijam, 1990 as in following:

Percentage of motility sperms	Description
0	Non motile
10-20	Some sperm are motile
30-40	High no. of non motile sperms

50-60	Half are motile
70-80	High no. of motile sperms
90-100	Most sperms are motile

2.9.3.2 Sperm Concentration:

Sperm concentration was calculated according to the following steps:

i. The sperm suspension prepared in (2.8) was pulled by the RBC pipette till "0.5" mark, and then diluted with the diluting solution till "101" mark, so the dilution rate is 1:200.
ii. The content of the pipette were mixed, the coverslip was placed over the counting chamber of the hemocytometer.
iii. A small amount of the diluting solution containing sperms was placed at the edge of the coverslip and drawn by the capillary action under the coverslip.
iv. The slide was placed under the microscope and the number of sperms was counted in five large squares.

The number of sperm / ml was calculated using the following formula:

$$\text{No.} = \frac{n * D*400*1000}{N*1/10}$$

n: no. of sperms in the five squares

D: inversion of the dilution rate (200)

400: inversion of the small square size

N: no. of small squares in the hemocytometer (80)

1 / 10: depth of the hemocytometer.

(Salisbury and VanDemark, 1961).

2.9.3.3 Percentage of Dead and Morphologically Abnormal Sperms:

The percentages of dead and morphologically abnormal sperms were measured as following according to the method reported by Bearden and Faquay, 1992:

1- A drop of the sperm suspension prepared in (2.8) was placed over the slide, and then a drop of eosin-nigrosin stain was added and mixed.
2- The mixture was spread using another slide and left to dry.
3- Using light microscope, 200 sperms were counted to calculate the percentage of dead sperms and morphologically abnormality of sperms as in the following equations:

$$\text{Percentage of dead sperms} = \frac{\text{No. of dead sperms}}{\text{Total sperm no.}} \times 100$$

$$\text{Percentage of morphologically abnormal sperms} = \frac{\text{No. of morphologically abnormal sperms}}{\text{Total sperm no.}} \times 100$$

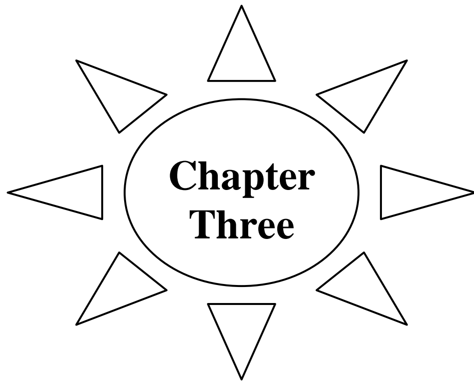
2.10 Tests for the *in vitro* Study:

The following three tests were done according to the methods reported by Breaden and Faquay, 1992 which are shown in (2.10.3):

1- Sperms motility.
2- Percentage of dead sperms.
3- Percentage of morphologically abnormal sperms.

2.11 Statistical Analysis:

The mean and the standard error of mean (SEM) were calculated, and the significance of difference analyzed by applying ANOVA test.



Results and Discussion

Results and Discussions

3.1 Plant Extracts:

3.1.1 Aqueous Extract:

Fifty grams of *Achillea millefolium* powdered flowers were used for preparation of this extract; the pH value for the aqueous extract was 5.5. The weight of the residue resulted after evaporation of distilled water was 10g, which represents 20% of the original flowers sample weight. This extract appeared with brown color.

3.1.2 Ethanolic Extract:

Fifty grams of *Achillea millefolium* powdered flowers were used for the preparation of this extract, the pH value for the ethanolic extract was 5.6. The weight of the residue obtained after evaporation of ethanol was 6.1g, which represents 12.2% of the original flowers sample weight. The appearance of the extract was dark brown in color.

3.2 Detection of Some Active Compounds in the Plant Extract:

The aqueous and ethanolic extracts of *Achillea millefolium* were subjected to chemical analysis to identify the compounds present in each extract.

The obtained results indicated the presence of flavonoids, glycosides, saponins and tannins in the aqueous extract, while the compounds contained in the ethanolic extract were alkaloids, coumarins, flavonoids, glycosides, resins, tannins and terpenes. Table (3-1)

Table (3-1): Chemical detection of some active compounds in *Achillea millefolium* aqueous and ethanolic extracts.

Chemical Compound	Reagent	Indication	Aqueous extract	Ethanolic extract
Alkaloids	Wagner's reagent Mayer's reagent Dragendorff's reagent	Brown ppt. White ppt. Orange ppt.	– – –	+ + +
Glycosides Before hydrolysis After hydrolysis	 Fehling reagent Fehling reagent	 Red ppt. Red ppt.	 + +	 + +
Flavonoids	Ethyl alcohol and potassium hydroxide	Yellow ppt.	+	+
Tannins	Lead acetate Ferric chloride	White gelatinous ppt. Greenish-blue ppt.	+ +	+ +
Resins	Hydrochloric acid	Turbidity	–	+
Coumarins	Sodium hydroxide	Greenish-yellow color	–	+
Terpenes	Acetic anhydride and sulfuric acid	Brown color	–	+
Saponins	Extract shaking Mercuric chloride	Foam White ppt.	+ +	– –

Note: +ve indicates the presence of the active compound.

-ve indicates the absence of the active compound

3.3 *In vivo* Effects of the Aqueous and Ethanolic Extracts:

3.3.1 Body Weight:

Doses of 200mg/kg/day of each extract were administered intraperitoneally in mice for 20 days. The body weight of mice was determined at the beginning of the experiment then body weight was measured weekly**.**

The results, which are given in the table (3-2), indicated that the same dose 200 mg/kg/day for 20 days of the aqueous and ethanolic extracts did not give any significant difference in the body weight during the experiment when compared with the control groups A, B and C, also no significant differences were obtained in body weight between control groups A, B and C as in table (3-2).

Similar results were obtained by Montanari *et al.*, 1998 in which *Achillea millefolium* ethanolic extract did not cause change in body weight of the male albino mice.

Table (3-2): *In vivo* effect of *Achillea millefolium* aqueous and ethanolic extracts (200mg/kg/day) for 20 days on body weight of mice.

Group (n=5)	Body weight (g)			
	Initial	First week	Second week	Final
Control A (untreated)	30.8 ± 0.8	30.25 ± 0.55	29.6 ± 1.6	29.45 ± 1.05
Control B (distilled water treated)	28.54 ± 1.54	27.58 ± 2.11	28.32 ± 2.45	27.96± 2.11
Control C (tween-20 and saline treated)	29.53 ± 0.9	27.23 ± 1.03	27.56 ±1.73	25.6 ± 1.81
Aqueous extract	31.36 ± 1.12	29.26 ± 1.29	30.56 ± 1.17	30.24 ± 1.27
Ethanolic extract	33.66 ± 1.18	33.02 ± 1.84	33.6 ± 1.81	30.98 ± 1.71

3.3.2 Testes Weights:

The possible effects of aqueous and ethanolic extracts were ascertained on the weight of testes. The obtained results after 20 days revealed that there was no significant difference in the mice testes weights following treatment with the aqueous and ethanolic extracts when compared with control groups A, B and C, also no significant difference obtained between control groups A, B and C as shown in table (3-3).

Similar results were obtained by Montanari *et al.*, 1998 in which *Achillea millefolium* ethanolic extract did not cause change in albino mice testes weights.

Table (3-3): *In vivo* effect of *Achillea millefolium* aqueous and ethanolic extracts at a dose of (200mg/kg/day) for 20 days on testis weight of mice.

Group (n = 5)	Testes weight (mg/100 g body weight)
Control A (untreated)	296.91 ± 1.87
Control B (distilled water treated)	282.65 ± 23.86
Control C (tween-20 and saline treated)	280.37 ± 27.91
Aqueous extract	296.77 ± 15.46
Ethanolic extract	288.82 ± 29.47

3.3.3 Sperm Functions:

The effect of these two extracts was studied on the followings:

3.3.3.1 Sperm Motility:

Determination of the percentage of motile sperm cells in the semen specimen is critical in the evaluation of semen (Ohl and Menge, 1996). The obtained results after injection of mice with the aqueous and ethanolic extracts for 20 day, revealed

that there was a significant decrease (P<0.05) in sperm motility of the aqueous extract treated group (44 ± 4.84) when compared with control group B (80 ± 1.58) and control group A (82.5 ± 2.5).

Also the reported results showed a significant decrease (P<0.05) in sperm motility after treatment with the ethanolic extract (59 ± 2.91) when compared with control group C (78.3 ± 1.66) and control group A (82.5 ± 2.5) table (3-4).

Moreover, the study revealed that there was a significant differences (P< 0.05) in sperms motility between the aqueous and ethanolic extracts. It is noteworthy to mention that there is no significant difference in sperm motility among control groups A, B and C as shown in table (3-4).

The aqueous extract was more effective in decreasing sperm motility than the ethanolic extract, which could be attributed to the action of saponins that were detected in the aqueous extract. The effect of these two extracts can be referred to the action of phenolic compounds, and to the terpenes in the ethanolic extract.

It had been reported that a phenolic compound, gossypol isolated from *Gossypium hirsutum* caused decrease in sperm motility in man (Prasad and Diczfalusy, 1982).

Zhen *et al*., 1995 had reported that six diterpenes isolated from *Tripterygium wilfordii* decrease sperm motility in man.

Other investigations reported that the ethanolic and aqueous extracts of other plants showed similar effects, Ashok and Meenakashi, 2004 had reported that *Curcuma longa* aqueous and ethanolic extracts decreases sperm motility in rats. Mali *et al*., 2002 showed that the ethanolic extract of *Martynia annua* reduces sperm motility in rats.

Sperm motility is considered as the most important parameter in determining fertilization rate (Mahadevan and Tronnson, 1984). Sperm specimen that exhibits decrease in motility may be competent to achieve fertilization but if the total motile sperm is decreased, the number of sperms reaching the upper females reproductive

tract may also be decreased (Ohl and Menge, 1996).

After transit of sperms through the epididymis, they acquire their potential for forward motility and fertilizing capacity (Hafez, 1975). Androgens play a role in determining the duration of survival of spermatozoa by exerting an effect either directly, on sperm cells or indirect by acting on the epididymis and its secretions (Nalbandov, 1976), so the epididymal epithelium is under control of androgens to provide essential factors that enable spermatozoa to develop full progressive motility (Moore *et al*., 1994; Kasturi *et al*., 1995).

It is well known that the secretion of proteins into the epididymal lumen influences sperm maturation, in which a glycoprotein present in the epididymal luminal fluid induces forward motility of the caput epididymal sperms (Palladino and Hinton, 1994). Therefore the decrease of sperm motility can be due to the alteration in secretion and function of proteins in the epididymis which caused a decline in sperm motility (Gupta *et al*., 2002), or may be due to inhibition of enzymes activity necessary for sperm metabolism (Lee and Moon, 1982).

Table (3-4): *In vivo* effect of Achillea *millefolium* aqueous and ethanolic extracts (200 mg/kg/day) for 20 days on sperm motility in mice.

Group (n = 5)	Sperm motility (%)
Control A (untreated)	82.5 ± 2.5 [a]
Control B (distilled water treated)	80 ± 1.58 [a]
Control C (tween-20 and saline treated)	78.3 ± 1.66 [a]
Aqueous extract	44 ± 4.84 [b]
Ethanolic extract	59 ± 2.91 [c]

[a,b,c] significant difference ($P<0.05$)

3.3.3.2 Sperm Concentration:

Measurements of sperm concentration have been used in the assessment of sperm functions (Motimer, 1994). The obtained results revealed that there was a significant decrease ($P<0.05$) in sperms concentration after treatment with the aqueous extract ($12*10^6 \pm 2$ sperm /ml) when compared with control group B ($24*10^6 \pm 2.44$ sperm /ml) and control group A ($25*10^6 \pm 4.08$ sperm /ml).

The data shown in table (3-5) revealed that a significant decrease ($P< 0.05$) in sperm concentration following treatment with the ethanolic extract ($16*10^6 \pm 2.44$ sperm/ml) as compared with control group C ($23.3*10^6 \pm 3.33$ sperm/ml) and control group A ($25*10^6 \pm 4.08$ sperm / ml), table (3-5).

There was a significant difference ($P<0.05$) between the aqueous and ethanolic extract treatment in which the aqueous extract was more effective in decreasing sperm concentration than ethanolic extract, but there was no significant difference in sperm concentration among control groups A, B and C, table (3-5).

The differences between the two extracts in decreasing sperm concentration are due to the differences in the activity of the active compounds that were detected in the aqueous extract and not detected in the ethanolic extract. The decrease in sperm after treatment with these two extracts can be referred to the action of flavonoids and phenolic compounds, and to the terpenes in the ethanolic extract.

A phenolic compound, gossypol isolated from *Gossypium hirsutum* caused oligospermia in human and animals (Parasad and Diczfalusy 1982; Zatuchni and Osborn, 1981). Flavonoids isolated from *Vitex negundo* showed a decrease in sperm concentration in males (Stylian, 1996).

Anderson, 2002 had reported that octaphenols and bisphenol A reduce sperm production in laboratory animals. Diterpenes from *Tripterygium wilfordii* were reported to cause oligospermia in man (Zhen *et al*., 1995).

Another investigation had reported that the aqueous and ethanolic extracts of *Curcuma longa* diminished the sperm concentration of the rats (Ashok and

Meenakashi, 2004). Reddy *et al*., 1995 had reported that *Hibicus rosa sinesis* ethanolic extract caused a decrease in sperm concentration in albino mice.

Androgens are essential for initiation and maintenance of spermatogenesis, LH, FSH and testosterone control spermatogenic process, so changes in the synthesis and metabolism of these hormones may result in the reduction in the number of sperms in the caudal epididymis (Sharma *et al*., 2003; Vijaykumar *et al*., 2004).

Table (3-5): *In vivo* effect of *Achillea millefolium* aqueous and ethanolic extracts (200mg/kg dose) for 20 days on sperm concentration in mice.

Group (n = 5)	Sperm concentration $*10^6$/ml
Control A (untreated)	25 ± 4.08 [a]
Control B (distilled water treated)	24 ± 2.44 [a]
Control C (tween-20 and saline treated)	23.3 ± 3.33 [a]
Aqueous extract	12 ± 2 [b]
Ethanolic extract	16 ± 2.44 [c]

[a,b,c] significant difference (P < 0.05).

3.3.3.3 Percentage of Dead Sperms:

Determination of the percentage of dead sperms is one of the important criteria in the assessment of sperm functions (Ohl and Menge, 1996). The obtained results revealed that there was a significant increase (P<0.05) in the percentage of dead (red color of sperm head due to staining by eosin) sperm after treatment with the aqueous extract (88 ± 2.77) when compared with control group B (14.8 ± 1.93)

and control group A (10.5 ± 1.41). Also a significant increase ($P<0.05$) in the percentage of dead sperms was observed after treatment with the ethanolic extract (66.6 ± 5.01) when compared with control group C (17.3 ± 2.02) and control group A (10.5 ± 1.41). Table (3-6).

There was a significant difference ($P<0.05$) in the obtained results of dead sperms' percentage between the aqueous and ethanolic extracts treated groups in which the aqueous extract effect was more than the ethanolic extract in increasing sperms' dead percentage, but there was no observed significant differences among control groups A, B and C. Table (3-6).

The aqueous extract contains compounds that are not detected in the ethanolic extract, so the higher effect of the aqueous extract can be referred to saponins.

The results showed that there was a decrease in the viability of sperms which reveals that the aqueous and ethanolic extracts contain compounds that affect the viable sperms, so the percentage of dead sperms was increased.

Viable sperms repel the vital stain (eosin-nigrosin), where as dead sperms have lost the structural integrity of their plasma membrane and therefore absorb the dye (Smikle and Turek, 1997).

The activity of the aqueous and ethanolic extracts can be referred to their content of flavonoidal and phenolic compounds.

Hypericin, a phenolic compound isolated from *Hypericum crispum* had showed an increase in the rate of dead sperms of male Swiss albino mice (Khalil, 1998). The laboratory experiments have been showed that cotton seed oil administration produced no viable sperms in rats (Zatuchni and Osborn, 1981). Farnsworth and Waller, 1982 had reported that flavonoids and phenolic compounds had a spermicidal activity.

Table (3-6): *In vivo* effect of *Achillea millefolium* aqueous and ethanolic extracts (200mg/kg/day for 20 days on the percentage of dead sperms of mice.

Group (n = 5)	Dead sperm (%)
Control A (untreated)	10.5 ± 1.41 [a]
Control B (distilled water treated)	14.8 ± 1.93 [a]
Control C (tween-20 and saline treated)	17.3 ± 2.02 [a]
Aqueous extract	88 ± 2.77 [b]
Ethanolic extract	66.6 ± 5.01[c]

[a,b,c] significant difference ($P < 0.05$).

3.3.3.4 Percentage of Morphologically Abnormal Sperms:

Morphological analysis of sperms is an important aspect in the assessment of sperm functions (Katz *et al.*, 1982). The obtained results revealed significant increase ($P< 0.05$) in the percentage of morphologically abnormal sperms after treatment with the aqueous extract (85.4 ± 4) when compared with control group B (10.2 ± 1.98) and control group A (7.5 ± 2.5). Also a significant increase ($P < 0.05$) was observed in the percentage of morphologically abnormal sperms after treatment with the ethanolic extract (73.2 ± 2.35) when compared with the control group C (12.6 ± 3.48) and with control group A (7.5 ± 2.5), table (3-7). The results showed that after treatment with the aqueous and ethanolic extracts for 20 days, morphological abnormalities of sperms were observed as in the figure (3-1).

There was a significant difference ($P < 0.05$) between the aqueous and ethanolic groups in which the aqueous extract was more effective in increasing the percentage of morphologically abnormal sperms than the ethanolic extract, but

there was no significant difference among control groups A, B and C, table (3-7).

The activity of these extracts can be referred to the phenolic compounds, but the difference in their effect is due to the divergence in types of the detected active compounds.

Khalil, 1998 had showed that, the phenolic compound hypericin from *Hypericum crispum* causes increase in the rate of abnormal sperms in male Swiss albino mice. Also another phenolic compound, gossypol from *Gossypium hirsutum* was reported to produce abnormal sperms (Zatuchni and Osborn, 1981).

Male reproductive function is under hormonal control. Spermatogenic process is under control of FSH and testosterone (Seeley *et al*., 1996). The formation of type A spermatogonia and conversion of primary spermatocyte into secondary spermatocyte (meiosis I) are dependant on testosterone while the final maturation steps of spermatids are dependant on FSH (Kiser *et al*., 1975; Arab *et al*., 1989; Ganong, 1991). The abnormal sperm morphology may reflect an abnormal intratesticular maturation (Acosta *et al*., 1988).

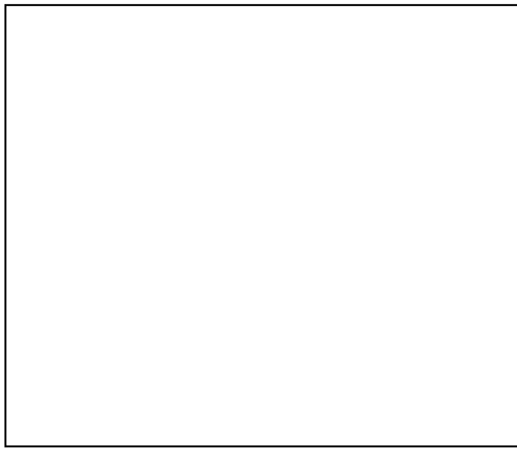
Drugs that induce an alteration in androgen secretion usually produce changes in the reproductive system, such changes might include the production of abnormal sperms (Tesarik *et al*., 1992), also any effect on spermatogenesis leads to the production of abnormal sperms (Arab *et al*., 1989). Low percentage of sperms exhibiting normal morphology have a greatly reduced progenesis for fertility (Kruger *et al*., 1986).

Morphologically normal sperm swim faster, straighter with higher tail beat frequencies than do morphologically abnormal sperm (Katz *et al*., 1982; Moralis *et al*., 1988).

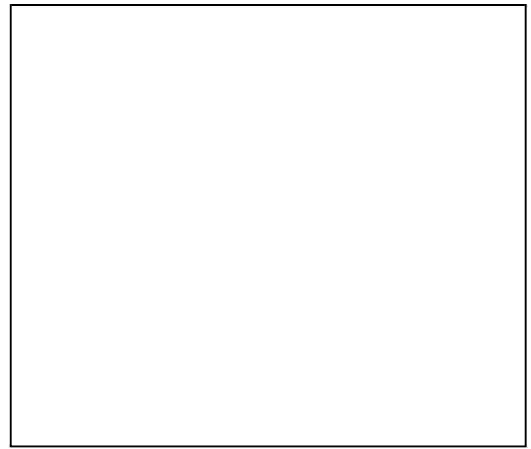
Table (3-7): *In vivo* effect of *Achillea millefolium* aqueous and ethanolic extracts (200mg/kg/day) for 20 days on the percentage of morphologically abnormal sperms in mice.

Group (n = 5)	morphologically abnormal sperms(%)
Control A (untreated)	7.5± 2.5 [a]
Control B (distil water treated)	10.2 ± 1.98 [a]
Control C (tween-20 and saline treated)	12.6 ± 3.48 [a]
Aqueous extract	85.4 ± 4 [b]
Ethanolic extract	73.2 ± 2.35 [c]

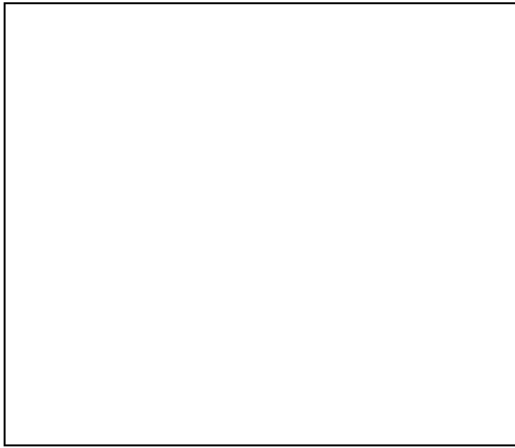
[a,b,c] significant difference ($P < 0.05$).



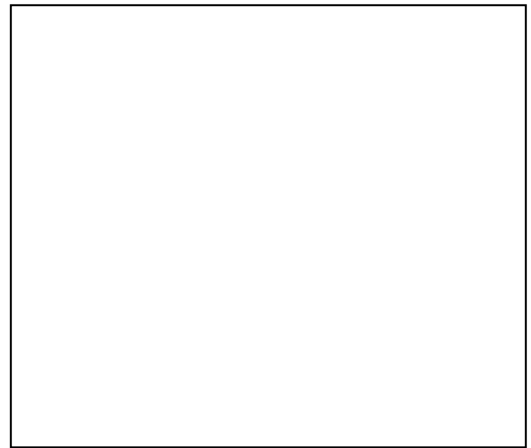
A- Normal sperm



B- Abnormal sperm



C- Bent sperm head



D- Broken sperm tail

E1- Broken sperm tail, E2- Bent sperm tail

Figure (3-1): *In vivo* Effects of *Achillea millefolium* aqueous and ethanolic extracts on sperm morphology in mice

3.4 *In vitro* Effect of the Aqueous and Ethanolic Extracts:

Standard sperm functions including sperm concentration, viability, motility and morphology are widely used as fundamental indicators of male fertility (Liu and Baker, 1992).

The aqueous and ethanolic extracts were added to the sperm suspension prepared in (2-8) at a concentration of 200 μg/ml, then examined after one minute and after 30 minutes of incubation at 37 ˚C. The observed results were recorded for some sperm functions as following:

3.4.1 Sperm Motility:

The results showed that sperm motility was affected by both extracts when used at 200 μg/ml. The obtained results which shown in table (3-8) gave a significant decrease (P< 0.05) in sperms motility after one minute after treatment with the aqueous extract (35 ± 2.88) in comparison with the control group (82.5 ± 2.5). A significant decrease (P< 0.05) in sperms motility was also observed after one minute after treatment with the ethanolic extract (51.66 ± 1.66) in comparison with the control group (82.5 ± 2.5).

The data obtained after one minute had showed a significant difference (P< 0.05) resulted between the aqueous and ethanolic extract in decreasing sperms motility, in which the aqueous extract was more effective than the ethanolic extract.

On the other hand, incubation of each extract with the sperm suspension for 30 minutes at 37 ˚C had come out of a significant decrease (P< 0.05) in sperms motility of aqueous extract treated sperm (10 ± 2.88) when compared with the control group (80 ± 3.16). For the ethanolic extract treated sperms, a significant decrease (P< 0.05) in sperms motility was observed (21.66 ±1.66) when compared with the control group (80 ± 3.16) table (3-9). There was a significant difference (P<0.05) in sperm motility between the aqueous and ethanolic extracts in which the aqueous extract was more effective than the ethanolic extract, due to the

compounds that were detected in the aqueous extract and not in the ethanolic extract. The decrease in sperm motility may be due to the action of alkaloids and terpenes.

It has been reported that azorellanone, a diterpene from *Azorella yareta* inhibit sperm motility in human *in vitro* (Moarles, *et al*., 2003). An alkaloid, berberine isolated from *Hydrastis canadensis* inhibited bull sperm motility *in vitro* (Sabir *et al*., 1978).

Similar result was obtained by Sur *et al*., 2002 for the decreasing sperm motility by *Aegle marmelos* aqueous extract.

Sperm motility is one of the sperm functions that are important to evaluate the semen, so if the percentage of motile sperms is decreased, the number of sperms reaching the upper female reproductive tract may be also decreased, thereby decreasing the chance for fertilization (Ohl and Menge, 1996). Inhibition of enzymes necessary for sperm metabolism will lead to decrease or loss the ability of the sperm motility (Lee and Moon, 1982).

Table (3-8): I*n vitro* assay on the effect of *Achillea millefolium* extracts (200µg/ml) on sperm motility in mice after one minute.

Treatment	Sperms motility (%)
Control (untreated)	82.5 ± 2.5 [a]
Aqueous extract	35 ± 2.88[b]
Ethanolic extract	51.66 ± 1.66 [c]

[a,b,c] significant difference ($P < 0.05$).

Table (3-9): I*n vitro* assay on the effect of *Achillea millefolium* extracts (200µg/ml) on sperm motility in mice after 30 minutes incubation.

Treatment	Sperms motility (%)
Control (untreated)	80 ± 3.16 [a]
Aqueous extract	10 ± 2.88 [b]
Ethanolic extract	21.66 ± 1.66 [c]

[a,b,c] significant difference ($P < 0.05$).

3.4.2 Percentage of Dead Sperms:

In this experiment both of the aqueous and ethanolic extracts were effective in increasing the percentage of dead sperms which are stained by eosin to have a red color heads. The obtained results after one minute had showed a significant increase ($P<0.05$) in the percentage of dead sperms after treatment with the aqueous extract (67.3 ± 3.92) when compared with the control group (10.5 ± 1.5). Table (3-10).

A significant increase ($P < 0.05$) in the percentage of dead sperms was observed after treatment with the ethanolic extract (49.33 ± 4.05) when compared with the control group (10.5 ± 1.5). Table (3-10)

The data obtained after one minute had showed a significant difference ($P< 0.05$) resulted between the aqueous and ethanolic extract in increasing percentage of dead sperms, in which the aqueous extract was more effective than the ethanolic extract.

After incubation of the sperm suspension with each one of these two extracts

for 30 minutes, the results showed a significant increase ($P<0.05$) in the percentage of dead sperms when incubated with the aqueous extract (95 ± 2.64) in comparison with the control group (11.5 ± 1.5). Table (3-11)
Also a significant increase ($P<0.05$) in the percentage of dead sperms was resulted when incubated with the ethanolic extract (81 ± 1.25) in comparison with the control group (11.5 ± 1.5). Table (3-11).

There was a significant difference ($P<0.05$) in sperms' dead percentage between the aqueous and ethanolic extracts in which the aqueous extract was more effective than the ethanolic extract after incubation.

These results indicated that both of the aqueous and ethanolic extracts have spermicidal activity *in vitro.*

The spermicidal activity of these extracts can be due to the action flavonoids and to the phenolic compounds, and to saponins in the aqueous extract.

It has been reported that the majority of plant-derived spermicides are saponins, flavonoids and phenolic compounds (Fransworth and Waller, 1982). The saponins of *Cyclomen persicum, Primula vulgaris* and *Gypsophyla paniculata* have been reported to cause almost instant immobilization of human spermatozoa (Primorac *et al*., 1985), also oleanolic acid from *Albizza procera* produce instant human sperm immobilization (Kamboj and Dhawan, 1982). Gossypol, a phenolic compound has a direct spermicidal activity (Waller *et al*., 1981).

Most of spermicidal compounds act on the plasma membrane of sperm lead to its destruction (Schill and Wolff, 1981 and Wilborn *et al*., 1983). Fransworth and Waller, 1982, had reported that saponins, flavonoids and phenolic compounds are responsible for this activity *in vitro,* so the activity of the aqueous and ethanolic extracts may be due to their content of the above mentioned active compounds.

Table (3-10): In *vitro* assay on the effect of *Achillea millefolium* extracts (200µg/ml) on sperms' dead percentage in mice after one minute.

Treatment	Percentage of dead sperms
Control (untreated)	10.5 ± 1.5 [a]
Aqueous extract	67.3 ± 3.92 [b]
Ethanolic extract	49.33 ± 4.05 [c]

[a,b,c] significant difference (P< 0.05).

Table (3-11): In *vitro* assay on the effect of *Achillea millefolium* extracts (200µg/ml) on sperms' dead percentage in mice after 30 minutes incubation.

Treatment	Percentage of dead sperms
Control (untreated)	11.5 ± 1.5 [a]
Aqueous extract	95 ± 2.64 [b]
Ethanolic extract	81 ± 1.25 [c]

[a,b,c] significant difference (P< 0.05).

3.4.3 Percentage of Morphologically Abnormal Sperms:

Morphological analysis of sperms is another important criteria to study the effect of drugs and chemicals (Hafez and Hafez, 2000). The aqueous and ethanolic extracts were tested after one minute. The results showed a significant increase (P<0.05) in the percentage of morphologically abnormal sperms when treated with

the aqueous extract (43.66 ± 3.75) when compared with the control group (7.5 ± 1.5). Moreover a significant increase ($P<0.05$) in the percentage of morphologically abnormal sperms resulted when treated with the ethanolic extract (31.3 ± 4.48) when compared with the control group (7.5 ± 1.5). Table (3-12). The data obtained after one minute had showed a significant difference ($P<0.05$) resulted between the aqueous and ethanolic extract in, increasing the percentage of morphologically abnormal sperms in which the aqueous extract was more effective than the ethanolic extract.

After incubation of these two extracts with the sperm suspension for 30 minutes, the obtained results had showed a significant increase ($P<0.05$) in the percentage of morphologically abnormal sperms after treatment with the aqueous extract (94.66 ± 1.76) in comparison with the control group (7.5 ± 1.5). Table (3-13). For the ethanolic extract, a significant increase ($P<0.05$) in the percentage of morphologically abnormal sperms after treatment of value (82.6 ± 2.9) in comparison with the control group (7.5 ± 1.5). Table (3-13).

There was a significant difference ($P<0.05$) in the percentage of morphologically abnormal sperms after incubation for 30 minutes between the aqueous and ethanolic extracts, in which the aqueous extract was more effective than the ethanolic extract, which is due to the differences in the types of active compounds detected in each of these two extracts. Various tail abnormalities were shown in figure (3-2).

Table (3-12): I*n vitro* assay on the effect of *Achillea millefolium* extracts (200µg/ml) on the percentage of morphologically abnormal sperms in mice after one minute.

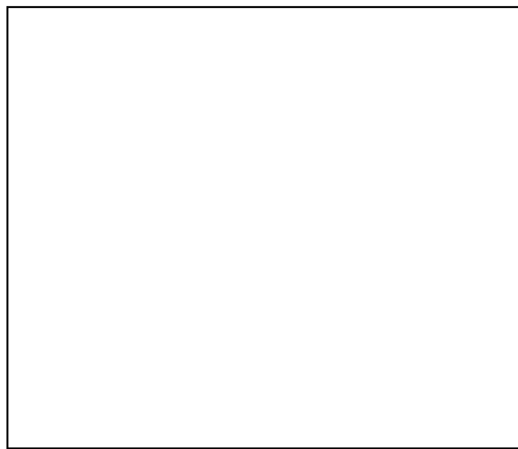
Treatment	Morphologically abnormal sperms' percentage
Control (untreated)	7.5± 1.5 [a]
Aqueous extract	43.66 ± 3.75 [b]
Ethanolic extract	31.3 ± 4.48 [c]

[a,b,c] significant difference ($P< 0.05$).

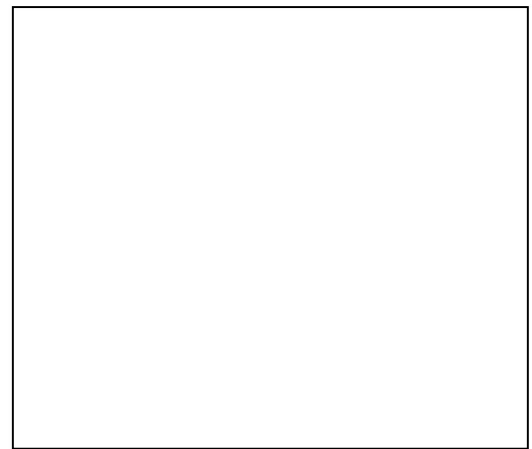
Table (3-13): I*n vitro* assay on the effect of *Achillea millefolium* extracts (200µg/ml) on the percentage of morphologically abnormal sperms in mice after 30 minutes incubation.

Treatment	Morphologically abnormal sperms' percentage
Control (untreated)	7.5 ± 1.5 [a]
Aqueous extract	94.66 ± 1.76 [b]
Ethanolic extract	82.6 ± 2.90 [c]

[a,b,c] significant difference ($P< 0.05$).



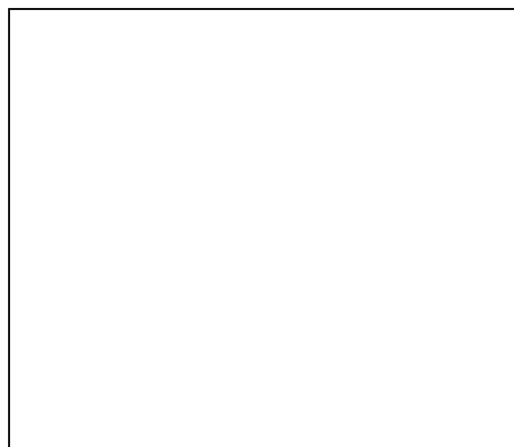
A- Normal sperm



B- Bent sperm head

C- Reflexed sperm tail

D- Bent sperm head



E- Broken sperm tail

Figure (3-2): *In vitro* effects of *Achillea millefolium* aqueous and ethanolic extracts on sperm morphology in mice

Conclusions

- Different active classes of compounds were detected in *Achillea millefolium* aqueous and ethanolic extracts, including alkaloids, glycosides, flavonoids, tannins resins, coumarins, terpenes and saponins.

- The aqueous extract was more effective than the ethanolic extract in both *in vivo* and *in vitro* tests, this was shown in decreasing sperm motility and increasing the percentage of dead and morphologically abnormal sperms, and decreasing of sperm concentration for *in vivo* test only.

Recommendations

- ☒ Further study on the purification of active compounds isolated from *Achillea millefolium* to identify the antifertility drugs.
- ☒ Further work on different pharmacological activities of *Achillea millefolium* active compounds including cytotoxicity and antitumor activities.
- ☒ Other experiments are required to examine the aqueous extract as spray on insects as insecticides to achieve male sterility in insects or as a pesticide.
- ☒ Histological study on the effects of different *Achillea millefolium* extracts on testes of male mice.
- ☒ Other studies on the effects of the aqueous and ethanolic extracts on body temperature, weight of thyroid gland, adrenal gland weight and accessory glands weights of the male reproductive system.
- ☒ Further studies on the effects of oral administration of the aqueous and ethanolic extracts on sperm function in male mice.
- ☒ Additional studies on the effects of roots and leaves extracts from *Achillea millefolium* on body weight, testes weight and on some sperm functions.

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Appendix A

Equipments

No.	Equipment name	Company / country
1	Autoclave	Dixons- UK
2	Balance	Ohaus- France
3	Electric grinder	Mammonlex- Taiwan
4	Incubator	GFL- USA
5	Laminar air flow cabinet	NAPCO- France
6	Light microscope	Olympus- Japan
7	Oven	Memmert- German
8	pH meter	PH M63-radio meter-Denmark
9	Sensitive balance	Mettler-Switzerland
10	Shaker incubator	GFl- USA
11	Vacuum pump	Robinair- USA
12	Vacuum rotary evaporator	BUCHI- Switzerland
13	Water bath	Memmert- German

Appendix B

Chemicals

No.	Chemical name	Company / Country
1	Acetic anhydride	Fluka- Switzerland
2	Bismuth subnitrate	Fluka- Switzerland
3	Chloroform	BDH-England
4	Eosin	Reidel-DE HaenAG seelze- Hanno-ven
5	Ethanol	Ferak- German
6	Ferric Chlorid	Fluka- Switzerland
7	Hydrochloric acid	Fluka- Switzerland
8	Iodine	BDH- England
9	Lead acetate	BDH- England
10	Mercuric chloride	Fluka- Switzerland
11	Nigrosin	LA- Chema- Russia
12	Potassium hydroxide	Fluka- Switzerland
13	Potassium iodide	BDH- England
14	RPMI-1640	Sigma -USA
15	Roshail salt	Fluka- Switzerland
16	Sodium citrate	Fluka- Switzerland
17	Sodium chloride	Fluka- Switzerland
18	Sodium hydroxide	Fluka- Switzerland
19	Sulphuric acid	Ferak – German
20	Tween-20	BDH- German

الخلاصة

تضمن البحث الحالي دراسة تاثير مستخلصين لازهار القيصوم على بعض وظائف الحيامن لذكور الفئران البيض داخل وخارج الجسم.

لقد تم تحضير مستخلصين من ازهار نبات القيصوم، المائي والايثانولي. واجري الكشف الكيميائي للتعرف على مجاميع المركبات الفعالة الموجودة في كل مستخلص وهي مركبات القلويدات والكلايكوسيدات والفلافونويدات والتانينات والراتنجات والكومارينات والتربينات والصابونيات.

تضمن البحث دراسة تاثير المستخلصين المائي والايثانولي داخل الجسم وذلك باجراء اختبارات وزن الجسم واوزان الخصى ودراسة بعض وظائف الحيمن المتضمنة الحركة والتركيز والعيوشية ونسبة التشوهات الشكلية.

تمت معاملة الحيوانات بهذين المستخلصين بجرعة 200 ملغم \ كغم \ يوم ولمدة 20 يوم. لم تبين النتائج اي تغيير في وزن الجسم ووزن الخصى، ولكن تبين وجود ضعف في حركة الحيامن وانخفاض تركيزها وزيادة في نسبة الحيامن الميتة والمشوهة شكلياً.

اجريت الدراسة خارج الجسم على تاثيرالمستخلصين المائي والايثانولي على بعض وظائف النطف المتضمنة: حركة الحيمن والعيوشية ونسبة الحيامن المشوهة شكليا كلا بعد مرور دقيقة واحدة وبعد مضي 30 دقيقة من الحضن،وقداستخدم تركيز 200 مايكرغرام \ مل لكل مستخلص. بعد مرور دقيقة واحدة اعطت النتائج انخفاضاً في حركة الحيامن وزيادة في نسبة الحيامن الميتة والمشوهة شكلياً وبعد 30 دقيقة من الحضن لوحظ نقصان اكثر في حركة الحيامن، وزيادة اكثر في نسبة الحيامن الميتة والمشوهة شكلياً.

دراسة تأثير مستخلصي ازهار نبات

القيصوم داخل وخارج الجسم على الخصوبة

في ذكور الفئران

رسالة مقدمة الى كلية العلوم في جامعة النهرين

وهي جزء من متطلبات نيل درجة ماجستير علوم في التقانة الاحيائية

من قبل

ضفاف يوسف يعقوب بطي

بكلوريوس تقانة احيائية 2001

جامعة النهرين

صفر ١٤٢٦

اذار ٢٠٠٥

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