Republic of Iraq
Ministry of Higher Education
and Scientific Research
Al-Nahrain University
College of Science
Department of Biotechnology

Effect of Aspirin on Male Hormones, Testicular and Epididymal Histology In Mice

A Thesis

Submitted to the College of Science Al-Nahrain University as a Partial Fulfillment of the Requirements for the Degree of Master of Science in Biotechnology

By

Bilal Salim Dawood Al-Tay

B.Sc. Biotechnology–Al-Nahrain University – 2004

November **2007**

Thelqeda **1428**

جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة النهرين
كلية العلوم
قسم التقانة الأحيائية

تـأثيرعقـــار الأسبرين على الـهرمونـات الذكرية وانسجة الخصى والبرابـخ في الفئـــــران

رسالة

مقدمة الى كلية العلوم - جامعة النهرين وهي جزء من متطلبات نيل درجة ماجستير علوم في التقانة الأحيائية

مِنْ قِبَلْ

بـــلال ســالم داؤد الطـــائي

بكالوريس تقانة احيائية – جامعة النهرين –٢٠٠٤

تشرين الثاني ٢٠٠٧

ذي القـعدة ١٤٢٨

List of Abbreviations

Abbreviations	Terms
ASA	Acetyl Salicylic Acid
ATP	Adenosine Triphosphate
BMP	Bone morphogenetic protein
COX	Cyclooxygenase
CVD	Cardiovascular disease
DNA	Deoxy Ribo Nucleic acid
EDTA	Ethylene Diamine Tetra Acetic acid
FSH	Follicle stimulating hormone
GnRH	Gonadotropin-releasing hormone
HCG	Human Chorionic Gonadotropin
iNOS	Inducible nitric oxide synthase
IVF	*Invitro* Fertilization
LH	Luteinizing hormone
NSAIDS	Non steroidal anti-inflammatory drugs
PGG/H	Prostaglandin G/H
PGH2	Prostaglandin H_2
PGI	Prostaglandin I
PGs	Prostaglandins
pKa	Dissociation Constant
TXA2	Thromboxane A_2

Acknowledgments

At the beginning, thanks to Allah who gave me the reality and strength to accomplish this work.

I would like to extend my thankful and appreciation to my supervisor Professor Dr. Adnan Salih Al-Janabi who have given me his advice and his important guidance throughout this work.

Also I would like to express my great thanks to the Dean of college of science Dr. Laith Al-Any, and to the head of Biotechnology Department of AL-Nahrain University Dr. Nabeel Al- Ani and my best gratitude to Dr. Khulood Al-Samarraee, Dr. Bassima Al-Jubori and Dr. Abd Alateef Al-Baitoshi for their support.

My sincere gratitude and deepest thanks and gratefulness goes to Dr. Hazim Al-Ahmed and Haider Abd Alzahra in the Biotechnology Research Center/ AL-Nahrain University for the statistical analysis of this work , laboratorial advise , and for there support and assistance. Special thanks, goes to my closest friends Waleed, Ossama and Creese.

Also I want to thank all my friends, especially Suhaib, Haider Mufeed, Hussain, Anas, Omar, Azad, Saddam, Moyasser, Taif, Nael, Akram, Salam, Mustafa, Dhilal and Ali, who helped me in finishing this work.

Finally, I would like to thank my family, and every one who gave a hand and encouraged me to complete this work.

Bilal

Committee Certification

We, the examining committee, certify that we have read this thesis and examined the student in its contents and that according to our opinion is accepted as a thesis for the degree of Master of Science in Biotechnology.

Signature:
Name: Prof. Dr. Alaa Al-Deen Ghalib Alkhalidy
Date:
(Chairman)

Signature:
Name: Prof. Dr. Waleed Hameed Yosif
Date:
(Member)

Signature:
Name: Ass. Prof. Mohammed Baker Mohammed Rashad
Date:
(Member)

Signature:
Name: Prof. Dr. Adnan Salih Al-Janabi
Date:
(Supervisor and Member)

I hereby certify upon the decision of the examining committee

Signature:

Name: Dr. Laith Abdul-Aziz Al-Ani

Scientific Degree: Assist. Prof.

Title: Dean of College of Science

Date:

Conclusions

From the results of the present study the following conclusions have been arrived at:

1. A significant decrease ($P < 0.05$) in body weight in experimental group 2 (with experimental group 1 and with control).

2. Aspirin administration concerning its effect on the male reproductive system caused a highly significant decrease in testicular and epididymal weight.

3. Aspirin administration also caused a significant decrease in seminiferous tubules diameter associated with increase in empty spaces between them and thickness of semeniferous tubules. A similar decrease in epidydimal tubular diameter and height of their cells.

4. A significant decrease in serum LH, FSH and testosterone level in both experimental groups.

CONTENTS

Subject **page**

CHAPTER TWO: MATERIALS AND METHODS

CHAPTER THREE: RESULTS AND DISCUSSION

CONCLUSIONS AND RECOMMENDATIONS

1. Introduction and Literature Review

1.1. Introduction

The first use of aspirin of the bark of the English willow in medicine was done by the Reverend Edmund stone in 1763 in the treatment of patients suffering from fever (Porter and Sturrock, 1993). Aspirin is an incredible chemical with many useful benefits in the medical field, it is a true herb, originally coming from the bark of the white willow tree (Katzung & Furst, 2001). It is an analgesic in coats blood platelet rendering them less likely to clot (Garella, 1988).One of the areas of aspirin effect on physiology of human and animals that has not been widely experimented on, is the area of its effect on the reproductive system male and female, and aspirin is still used by human for its various mentioned health problems studies on its reproductive effect is very vital to avoid unnecessary side effect (Ferrira *et al*., 1971). It has been used for years by heart patients to prevent clots from forming on known cholesterol plaques in the heart arteries, it also can prevent heart attacks in otherwise healthy people (Frank and Jackson, 1998). In a large, very well conducted study, 25,000 doctors took an aspirin tablet one day and a beta carotene capsule the next, results indicated that beta carotene (the widely known anti-oxidant) was ineffective in preventing cancer. In fact, it seemed to increase the chance of lung cancer in cigarette smokers. On the other hand, those physicians taking an aspirin tablet every other day had half the heart attacks as those who did not (Laurence, 1997).

History of peptic ulcers or a bleeding problem, aspirin may not be useful and only very low dose of aspirin each day may be used (Goodman and Gilman, 1996). Aspirin now is widely used all over the globe not only as antipyretic but also anti-inflammatory drug. Until the late 1970s the use of low dose aspirin was limited to the treatment of angina, stroke, myocardial

infarction, cerebrovascular ailments, anti-tumor immunity and many non pregnancy-related disorders (Lenz and Wilson, 2003). Later on aspirin has been found to be analgesic in addition to its antipyretic effect and in small dose it has a powerful effect as an anti-inflammatory and pain-reliever (LoLL *et al*., 1995).

In 1853, a French chemist, Charles Frederic tried to improve on the sodium salicylate mixture by combining it with acetyl chloride, and he succeeded in producing a compound that was less irritating to the stomach (Levesque and Lafout, 2000).

The objectives of the present study which performed on male mature mice (as an experimental model) were:

1. Effect of its administration of low and high dose on spermatogenesis.

2. Various changes associated with spermatogenesis viz: structural testicular and epididymal changes, reproductive hormonal changes.

1.2. Literature Review

1.2.1. Historical Account of Aspirin

Human beings since earlier times in history searched for substances that have the ability to reduce pain and fevers in their bodies. In these endeavors, leaves, roots, fruits, pollens of plants were tried and different human cultures in various areas in our globe developed their own medicine including agents that could cure or reduce pain. In this respect Edmund Stone wrote a letter to the Royal society in 1763, describing his success in treating fever with a powdered from the bark of the Willow (Katzung and Furst, 2001). Aspirin has been used as a pain reliever for more than 100 years, including headache, fever or for arthritis pain. But this common medication has a wider range of uses. Aspirin can help in preventing heart attacks and strokes and might even reduce your risk of some cancers (Rice *et al*., 2004).

Since 600 B.C., healers have used willow tree bark, which contains an aspirin-like substance, to treat common ailments. In the 1890s, scientist patented the first production of aspirin, whose chemical name is acetylsalicylic acid (Bayer, 2004). Aspirin quickly became popular to reduce pain (Porter and Sturrock, 1993). It inactivates blood clotting by platelets. It also blocks an enzyme called (COX), which helps make chemicals called prostaglandins that are involved in pain, inflammation, and possibly in regulating cell development (Zangwill *et al*., 2004).

Aspirin is the prototype of nonsteroidal anti-inflammatory drugs (NSAIDS); the quantities of aspirin consumed in various countries reach a level of thousands of tons annually. In the United States, for instance reports indicate figure of up to 10,000 to 20,000 tons annually, the origins of aspirin date back over 3500 years. The Eberus papyrus (a collection of medicinal recipes dating back to the middle of the second millennium BC) describes an

infusion of dried myrtle leaves used to ease the pain of rheumatism and for back problems (Al-Bayati, 2002).

The "A" comes from acetyl chloride; the "SPIR" comes from spiraea ulmaria, the plant they used to obtain the salicylic acid from and "IN" was a common ending for medicines at that time (Vane and Botting, 2003).

Juice extracted from the bark of willow trees was later used by Hippocrates, the celebrated ‘father of medicine', in the fifth century B.C. to ease pain and fevers. Both of these preparations contain salicylic acid, the precursor of modern aspirin (Goodman and Gilman's, 1996).

Salicin, the parent of the salicylate drug family, was successfully isolated in 1829 from willow bark, sodium salicylate, a predecessor to aspirin, was developed along with salicylic acid in 1875 as a pain reliever (the Medical team of Apollo health Street, 2001) (Brenneck *et al*., 1995).

1.2.2. Chemistry of Aspirin

Aspirin when exposed to moisture it hydrolyse into salicylic acid and acetic acid, and give off a vinegary-odor. It is highly lipid soluble and slightly soluble in water (Anonomous, 1999). Most molecules are made of more than one kind of atom. An aspirin molecule is made up of carbon atoms, hydrogen atoms, and oxygen atoms. As shown in figure (1), (William, 1995).

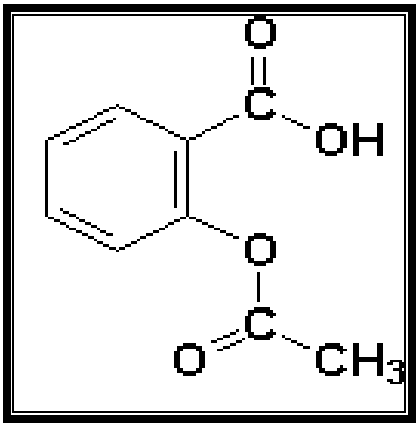


Figure 1: The amazing aspirin molecule (William, 1995**).**

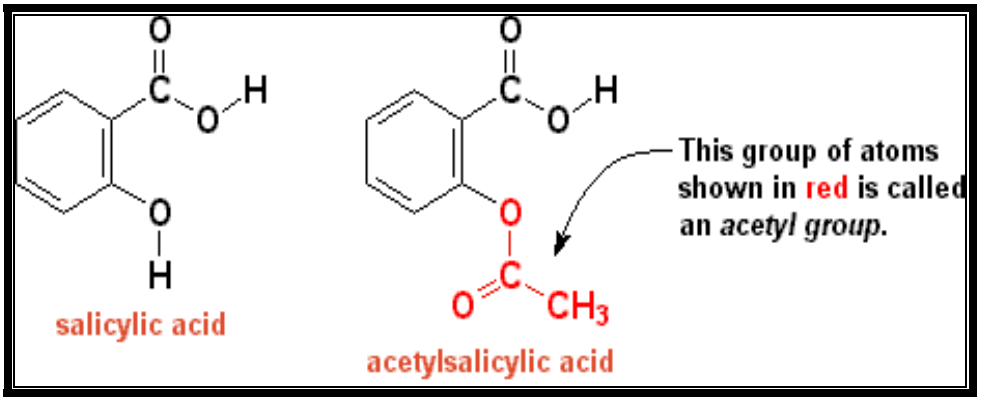


Figure 2: Formation of Acetyl Salicylic acid from salicylic acid
(Stillings *et al*., 2000).

Since Felix Hoffmann added a group of atoms to the salicylic acid molecule that we call an acetyl group, the new molecule is called acetylsalicylic acid (Al-Bayati, 2002).

Aspirin as mentioned earlier falls into a category of drugs called (NSAIDS), which also includes ibuprofen, and naproxen sodium. NSAIDS are unique in their ability to reduce swelling, unlike other drugs, such as acetaminophen (Tylenol), which relieve pain, but not inflammation (Rice *et al*., 2004). The drug is an ester of acetic acid; in addition there are salts of salicylic acid. The chemical relationships can be seen from the structural

formulas in the figure (2). There is a relationship between structure and activity, salicylatcs generally act by virtue of their content of salicylic acid, and some of the unique effects of aspirin are due to its capacity to acetylate proteins. Substitution on the carboxyl or hydroxyl group changes the potency or toxicity of salicylate agents (Carey, 1987). Salicylic acid (orthohydroxy benzoic acid) is so irritating that can be used only externally, therefore various derivatives of this acid have been synthesized for systemic use, these comprise two large classes, namely esters of salicylic acid obtained by substitution in the carboxyl group and salicylate esters of organic acids in which carboxyl group of salicylic acid is retained and substitutions made in hydroxyl group (Jones, 2001).

1.2.3. Metabolism of Aspirin

Salicylic acid is primarly conjugated in the liver to form salicyluric acid, phenolic glucuronide, and a number of minor metabolites. When the hepatic pathway becomes saturated, zero-order kinetics prevails, the drug having a half life of 15 hours or more (Verbeeck, 1990). Aspirin is rapidly metabolized, resulting in peak concentrations of salicylate in the plasma and exudates that exceeded peak concentrations of aspirin by 30- to 50-fold. Furthermore, concentrations of aspirin rapidly declined, whereas high concentrations of salicylate persisted in the plasma and in the exudates for up to 6 hr after a single administration of aspirin (Higgs *et al*., 1999).

Biotransformation of salicylates occurs in the microsomal-drug metabolizing system, and the following the catabolic products arc seen in the urine: Salicyluric acid (75%), phenolic glucuronide (10%),: acylglucuronide (5%), and free salicylic acid (10%). When hepatic pathway becomes saturated, zero-order kinetics prevail with a drug half-life of 15 hours or more (Verbeeck, 1990). The rate of urinary excretion is higher in alkaline than in

acidic urine, since more of the unchanged salicylate will be ionized and therefore less will be reabsorbed in the tubules (Gordon, 1984; Henry and Volans, 1984; Range *et al*., 1999).

The effects of chronic administration of aspirin in therapeutic doses (3.9 g/day) on plasma and salivary salicylate levels were studied, plasma and salivary salicylate levels declined significantly after peak levels were achieved between days 3 and 10. The decline in plasma and salivary salicylate levels may be due to an induction of a metabolic pathway such as salicylurate formation, Only the mean fraction of salicylate excreted as salicylurate appears to increase, the decline in plasma and salivary salicylate levels during chronic therapy may lead to an apparent 'tolerance' of some rheumatoid patients to aspirin (Rumble *et al*., 1980).

At therapeutic levels, 90% of salicylate is protein bound and therefore limited to the vascular space; the drug is then partially glycenated in the liver to salicyluric acid, which is both less toxic and more rapidly excreted by the kidney than salicylate (Jacob, 1996; Garella, 1988).

1.2.4. Mechanism of Action of Aspirin

As been mentioned aspirin and its beneficial effects on fever and pain were discovered more than two centuries ago and yet the mechanism of its medical action remains unclear until the late sixties of the 20^{th} century (Sneader, 2000). Aspirin inhibits the enzyme COX; COX is the enzyme which catalyzes the conversion of arachidonic acid to endoperoxide compounds (Meade *et al*., 1993). NSAID use continues to expand at a remarkable rate due both to the broad spectrum of clinical applications for these medications and to the relatively recent introduction of the popular COX-2-selective inhibitors (Harder *et al*., 2005).

Aspirin inhibits PGs synthesis in different tissues by (60-90%) (Horton *et al*., 1991). When aspirin is given in an appropriate dose to human (75-150 mg/day), it decreases the formations of both PGs and TXA2 but not the leukotriens (Abou and Alwan, 1990). Aspirin inhibits the first step in the coagulation of blood- aggregation of platelet (Bhajat, 1994). Aspirin prevents TXA2 formation by irreversibly inhibiting the enzyme COX (Blanco *et al*., 1999). Aspirin doses as low as 75 mg/day can have this effect (Goodman and Gilman, 1995). A single dose of aspirin inhibits the platelet cyclooxygenase for the life time of the platelet (8-11 days) (Schafer, 1999). It has been hypothesized that, for higher doses actually might reverse this tendency due to concomitant inhibition of endothelial cell synthesis of prostacylin, a vasodilator and inhibitor of platelet aggregation (Cerletti *et al*., 2003).

There are two isoformes of the COX enzyme, isoenzyme of PGG/H synthase) type 1 is constitutively expressed in most tissues and platelets and is involved in the synthesis of PGs and TXA2 that participate in maintaining cellular functions such as regulating interactions between platelets and endothelium (Patrignani *et al*., 1995). Aspirin selectively acetylates the hydroxyl group of a single serine residue at position 529 within the polypeptide chain of human platelet PGG/H synthase 1, causing the irreversible loss of it's COX activity, then the results of decreased conversion of archidonate to PGG2 and ultimately decrease PGH2 and TXA2, since they are synthesized from PGG2 (Funk *et al*., 1991). Normal physiological function appears to be maintained by COX-1, while, COX-2 appears to mediate the inflammatory response, NSAIDs with selective inhibitory activity on the COX-2 isoform which should theoretically decrease the inflammation while maintaining normal physiological PGs levels (Vane *et al*., 1998).

Aspirin is rapidly deacetylated by estrases in the body producing salicylate, which has anti-inflammatory, antipyretic and analgesic effects, the

anti-inflammatory and antipyretic effects of salicylates are primarily due to the blockade of PGs synthesis at the thermoregulatory centers in the hypothalamus and at peripheral target sites (Lippincott, 2000). Aspirin prevents the sensitization of pain receptors to both mechanical and chemical stimuli, and depressing pain stimuli at the subcortical site (i.e.: the thalamus and hypothalamus) (Makino *et al*., 1974).

Aspirin will not only inhibits COX-2 expression but will also suppress other genes involved in the inflammatory response, tumor growth and tissue injury, recently it has been reported that, aspirin and sodium salicylate at therapeutic concentration inhibited the activation of COX-2 promoter, reduced new mRNA synthesis and suppressed protein expression (XU *et al*., 1999). An example of the modification of aspirin-Like Drugs: effect on Inducible nitric oxide synthase, Nitric oxide synthesized by inducible nitric oxide synthase has been implicated as a mediator of inflammation in rheumatic and autoimmune diseases.

1.2.5. Effect of Aspirin on Reproductive System

Dosing duration should be increased relative to the intended therapeutic use; one approach to consider is establishing exposure and initial tolerability in a dose-range finding study followed by a definitive study powered to assess specific concerns (Beckman *et al*., 2003). Vane showed that aspirin and the compounds that have become NSAIDs, such as indometacin, inhibit the production of prostaglandins from arachidonic acid by inhibiting the enzyme COX1 (Flower, 2006).

1.2.6. Pharmacological Effects of Hormones

Administration of very large quantities of a hormone for medical purposes may have effects that are never seen in a normal person, hormonal mechanisms that influence the male reproductive system involve the hypothalamus of the brain, the anterior pituitary gland and the testis (Arthur *et al*., 1998). 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, regulation of hormonal status is achieved throughout feed-back mechanism, both inhibin and activin acting as a feedback regulators of FSH release (Lin *et al*., 1989).

1.2.7. Pituitary Gland

The pituitary gland is a small gland about the size of a pea in human (Patrono, 2001). It rests in a depression of the sphenoid bone inferior to the hypothalamus of the brain. The hypothalamus is an important autonomic nervous system and endocrine control center of the brain (Seeley *et al*., 1996). The pituitary gland originates through the interaction of two different ectodermal tissues, the neural and oral ectoderm, the neural ectoderm gives rise to the posterior pituitary, whereas part of the oral ectoderm will develop into the anterior and intermediate pituitary gland containing at least six distinct cell phenotypes, recent work has begun to unravel general mechanisms of pituitary organ induction based on defining the obligatory interactions between the neural and oral ectoderm as a prerequisite for pituitary gland formation (Treier *et al*., 2001**).**

1.2.8. Hormones of the Anterior Pituitary

The anterior pituitary is made up of epithelial cells derived from the embryonic oral cavity, and its hormones include:-

Growth hormone, Thyroid-stimulating hormone (TSH), Melanocyte-stimulating hormone (MSH), Luteinizing hormone (LH), Follicle- stimulating hormone (FSH) and Prolactin (Seeley *et al*., 1996)**.** Secretion of the anterior pituitary hormones is largely regulated by hormones produced by the hypothalamus and collectively called hypophysiotropic hormones, these hormones are secreted by neurons that originate in diverse areas of the hypothalamus and terminate in median eminence around the capillaries that are the origins of the hypothalamo-pituitary portal vessels (Arthur *et al*., 1998).

1.2.8.1. Luteinizing Hormone (LH)

The target tissues of LH are testis in males and ovary in females. It promotes testosterone synthesis and support for sperm cell production in testis. Ovulation and progesterone production in the ovary (Seeley *et al*., 1996). In both males and females, LH is essential for reproduction, in females, at the time of menstruation, FSH initiates follicular growth, specifically affecting granulosa cells, with the rise in estrogens, also LH receptors are expressed on the maturing follicle that produces an increasing amount of estradiol, eventually at the time of the maturation of the follicle, the estrogen rise leads via the hypothalamic interface to the “positive feed-back” effect, a release of LH over a 24-48 hour (Arthur, 1998). In both sexes, LH stimulates secretion of sex steroids from the gonads. In the testes, LH binds to receptors on Leydig cells, stimulating synthesis and secretion of testosterone. Theca cells in the ovary respond to LH stimulation by secretion

of testosterone, which is converted into estrogen by adjacent granulosa cells, in females, ovulation of mature follicles on the ovary is induced by a large burst of LH secretion known as the preovulatory LH surge **(**Bowen, 2004**).** LH stimulates the leydig cells to secrete testosterone, it's some times given the name interstitial stimulating hormone because it controls the production of testosterone by the interstitial cells which are scattered in the spaces between somniferous tubules (Mader, 1993).

1.2.8.2. Follicle-stimulating Hormone (FSH)

The target tissues of FSH hormone are seminiferous tubules in males and follicles in ovary. It promotes sperm cell production in testis, follicle maturation and estrogen secretion in ovary (Seeley *et al*., 1996). Pituitary gonadotropins FSH and LH hormones stimulate the gonads by regulating germ cell proliferation and differentiation. FSH receptors localized to testicular Sertoli cells and ovarian granulosa cells and are coupled to activation of the adenylyl cyclase and other signaling pathways **(**Dierich *et al*., 1998**).** FSH stimulates the maturation of ovarian follicles, administration of FSH to humans and animals induces superovulation or development of more than the usual number of mature follicles and hence, an increased number of mature gametes. FSH is also critical for sperm production, it supports the function of Sertoli cells, which in turn support many aspects of sperm cell maturation (Bowen, 2004). FSH stimulates the Sertoli cells in the seminiferous tubules to facilitate sperm development and to secrete inhibin and activin, which regulates the FSH secretion (Mader, 1993).

1.2.9. Hormones of the posterior pituitary

Posterior pituitary is an extension of the brain and is made up of nerve cells, and its hormones include:-

Antidiuretic hormone which increase water reabsorption, and Oxytocin which increase uterine contractions (Seeley *et al*., 1996), Vasopressin and oxytocin are also produced in other areas of the brain and serve in those sites as neurotransmitters or neuromodulators (Arthur *et al*., 1998).

1.2.10. Testis

The testes of the male secrete sex hormones, in addition to producing sperm cells. The main hormone produced is testosterone. It is responsible for the growth and development of the male reproductive structures, muscle enlargement, growth of body hair and increase male sexual drive (Seeley *et al*., 1996). The contractile "myoid cells" surrounding the seminiferous tubules serve as a barrier to the penetration of substances into the germinal epithelium (Dym *et al*., 1970). The efferent ductules lead from the testes to a tightly coiled series of threadlike tubules that form a comma-shaped structure on the posterior side of the testes called the epididymis, within the epididymis, sperm cells develop the capacity to swim and the ability to bind to the secondary oocyte (Seeley *et al*., 1996).

1.2.10.1. Testosterone

Testosterone is the major male hormone secreted by the testes, during puberty, testosterone causes the enlargement and differentiation of male genitals and reproductive duct system, is necessary for spermatogenesis, and encourages the development of male secondary sexual characteristics (Seeley *et al*., 1996). In addition to its essential paracrine action within the testes on

spermatogenesis and its negative-feedback effects on the hypothalamus and anterior pituitary, testosterone is essential in males for development of sex drive at puberty, and in the adult male (Arthur, 1998). Testosterone therapy for hypogonadal male should correct the clinical abnormalities of testosterone deficiency, including improvement of sexual function, increase in muscle mass and strength, and decrease in fat mass, with minimal adverse effects, administration of a new transdermal testosterone gel formulation to hypogonadal male provided dose proportional increases in serum testosterone levels to the normal adult male range **(**Wang *et al*., 2000**).**

Testosterone administration to older male improves muscle function: molecular and physiological mechanisms, a significant decrease in serum total testosterone occurs as early as ages and this decrease in testosterone production is associated with the loss of lean body mass and muscle strength (Ferrando *et al*., 2001). It was demonstrated that testosterone administration primes skeletal muscle for growth by increasing net protein synthesis in the fasted state, the logical extrapolation of a continued increase in net protein synthesis is an increase in lean body mass and strength (Abbasi *et al*., 1993).

Testosterone is necessary for proper sperm production, development and maintenance of male reproductive organs and stimulates development of male secondary sexual characteristics; testosterone has a negative feed-back effect of the hypothalamus and pituitary gland to reduce LH and FSH secretion (Raven and Johanson, 1996).

It was demonstrated that supraphysiological doses of testosterone can induce increases in muscle size and strength in younger male without concomitant fatigue, this relationship holds true in relatively hypogonadal populations, where the increase of circulating testosterone increases muscle protein synthesis and muscle strength, in an earlier study (Bhasin *et al*., 1993). It was demonstrated that one of the primary effects of testosterone is

the efficient reutilization of intracellular amino acids which derived from protein breakdown for protein synthesis, however, it was demonstrates that, even if breakdown is decreased, amino acid precursors are present to support the initial rate of protein synthesis, thus testosterone administration may ameliorate the loss of skeletal muscle nitrogen during fasting in this older population by preventing the loss of intracellular amino acids (Morley *et al*., 1993).

1.2.11. Sperm

The entire process of sperm formation, beginning with spermatogonia and resulting in mature spermatozoa is referred to as spermatogenesis; this occurs in the seminiferous tubules, the seminiferous epithelium lining the seminiferous tubule is compared of two basic cell types: the developing germ cells and the Sertoli cells (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, 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 from primary spermatocytes (Saladin *et al*., 1998).

The sites of sperm formation, or spermatogenesis, in the testes are the many tiny, convoluted seminiferous tubules, movement of the sperm as far as the epididymis results from the pressure created both by the continuous formation of fluid by the Sertoli cells back in the seminiferous tubules and by peristalsis of the tubules (Arthur, 1998). The semeniferous tubules contain germ cells and Sertoli, which are large cells that extend from the periphery to

the lumen of the seminiferous tubule, they nourish the germ cells and probably produce a number of hormones, germ cells are scattered between the Sertoli cells (Seeley *et al*., 1996).

Supplementation with carnitine improves sperm quality and/or quantity in testes of mice exposed to physical insults, such as heat and X-irradiation, and in men with idiopathic oligoasthenospermia. These benefits may be due to increased mitochondrial fatty acid oxidation resulting in improvement in motility of epididymal sperm (Ng *et al*., 2004). Semen is known to be an important vector in the dissemination of viral diseases, and testing for viruses in the testis has not been extensive despite the possibility that they are involved in some of the disorders of this organ, such as orchitis, decrease in semen quality, azoospermia, and testicular carcinoma (Dejucq *et al*., 1998). An important indicator of sperm maturity and reproductive health is sperm shape, which is directly related to cytoplasmic retention and the spermiogenetic maturation process (Huszar and Vigue, 2004).

The degree of sperm DNA strand fragmentation is a very important measure of sperm function because DNA integrity plays a key role in sperm fertilizing potential and in providing the parental contributions of the sperm to zygote development (Manicardi *et al*., 1995; Sakkas *et al*., 1999; Irvine *et al*, 2000 and Ward *et al*., 2003).

Sperm DNA fragmentation, which is also related to sperm maturity, is also a reflection of the level of reactive oxygen species in sperm (Aitken *et al*, 1994; Huszar and Vigue, 1994). Studies directed to male infertility or to could be improved if, in addition to the conventional sperm concentration and motility parameters, validated biomarkers of sperm maturation and function were also utilized (Krausz *et al*., 1994). It is also expected that semen collection in the home environment will increase participation compared with semen collection at an impersonal study trailer or laboratory, In addition,

analysis in a centralized laboratory will enhance quality control, which is important in the monitoring of male who are exposed to or removed from the adverse environment of reproductive toxicity (Irvine *et al*., 1997).

In these cases, the sperm concentration or motility might remain similar, but the biochemical markers should demonstrate the adverse or beneficial effects, respectively (Ward *et al*., 2003). Mature mouse oocytes are arrested at metaphase of the second meiotic division. Completion of meiosis and a block to polyspermy is caused by a series of repetitive Ca^{2+} transients triggered by the sperm at fertilization (Jones *et al*., 1995). Repetitive Ca^{2+} transients have been observed during the fertilization of mammalian oocytes (Cuthbertson *et al*., 1981; Cuthbertson and Cobbold, 1985; Igusa and Miyazaki, 1986; Kline and Kline, 1992 and Taylor *et al*., 1993).

There is a correlation between birth weight and sperm counts in adult life were anticipated because impaired fetal growth could impair replication of Sertoli cells produced in fetal life. Furthermore, it was expected that males born with a high birth weight might have impaired sperm production as they are expected to have been exposed to higher levels of estrogen in fetal life (Krause *et al*., 2001).

Studies indicate that sperm longevity decreases with sperm size (Parker, 1993). Faster-swimming sperm may be advantageous if there is active competition among sperm at the site of fertilization, while it seems logical that there would be a correlation between sperm swimming speed and fertilization success, this idea has not been tested (Gage *et al*., 1995).

Swimming speed must be influenced by some other factor such as energy (ATP) stores (Billard *et al*., 1995). If there is a positive relationship between sperm length and swimming speed, as has been suggested for mammals (Gomendio and Roldan 1991), males in better condition would be at a competitive advantage in sperm competition, recent evidence, however,

suggests that there is no interspecific relationship between sperm length and swimming speed in birds or Pacific salmon (Leach, 1997).

1.2.11.1. Spermatozoon Characteristics

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 membrane**s** and covers the anterior part of the sperm nucleus, it contains enzymes, important in penetration of the egg in the fertilization process, 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 surrounded by nine coarse fibers which is in turn surrounded by the mitochondrial sheath (Saladin *et al*., 1998).

1.2.12. Clinical Uses of Aspirin

The chronic administration of aspirin and other NSAIDS may interfere with the hydrolysis of phospholipids, since may diminish the loss of neurons, these agents may beneficially affect the development of Alzheimer's disease (Square, 1997). Numerous measures have been employed in an attempt to increase implantation and pregnancy rates in assisted reproduction, aspirin has been utilized as one such potential therapy. This drug has been shown to increase uterine blood flow, aspirin could improve the receptiveness of the endometrium, thereby increasing implantation and birth rates (Hurst *et al*., 2005). Some patients with placental insufficiency or preclampsia, and venous thrombosis may benefit from the antiplatelet effect of aspirin, as suggested by individual trials or overviews (Hirsh *et al*., 1992).

Low-dose aspirin treatment (100mg/day) improves ovarian responsiveness, uterine and ovarian blood flow velocity, implantation and pregnancy rates in patients undergoing IVF, higher pregnancy rate and better endometrial pattern achieved in patients with thin endometrium alter low-dose aspirin administration (Hsieh- Yiy *et al*., 2000).

Clinical studies indicate that, the use of NSAIDs for short period of time (3 days) yield good results and does not result in intrauterine closure of the ducts arteriosus and pulmonary hypertension, treatment with low-dose aspirin (50mg/day) resulted in improved birth weight and lower rates of still births, abrupt placenta and intrauterine growth retardation Aspirin should be avoided 1 week prior to and during labour because it can result in excessive blood loss (Wallenburg and Rotmans, 1987; Trudinger *et al*., 1988).

Aspirin in low dose is effective in the prevention and treatment of cardiovascular disease and increased the weight of new born in pregnant patients with fetal growth retardation, it also used to prevent idiopathic fetal growth retardation and to improve placental and fetal blood flow in women with pre-eclampsia, regarding the dose determination, the effect of a range of a very low-dose of aspirin (40-324mg/day) on TXA2 and prostacyclin (PGI_2) have been evaluated (Lorenz *et al*., 1989).

All doses of aspirin suppressed TXA_2 formation by > 80%, and suppression of PGI_2 formation was more pronounced with (324mg) of aspirin as compared with other doses, low-dose of aspirin seems to be more platelet selective than high doses with effect of about 100mg/day (Weber *et al*., 2002).

1.2.13. Male Reproductive System

The male reproductive system consists of the primary reproductive organs, the testes, and the secondary reproductive organs, which include the scrotum, epididymis, ductus deferens, seminal vesicles, urethra, prostate gland, bulbourethral glands, and penis (Seeley *et al*., 1996). The role of androgens in the development of male reproductive tissues and reproductive performance is well characterized, estrogens are known to be involved in the negative feedback regulation of gonadotropin secretion in men, and are important for the masculinization of the male brain during development and for the maintenance of sexual behavior during adulthood **(**Li *et al*., 2001**).** The bone morphogenetic protein (BMP) system plays a crucial role in fertility in female and male mammals, Availability of recombinant BMPs has enabled functional studies that have demonstrated important biological activities of BMPs in controlling cellular proliferation, differentiation, and apoptosis in reproductive tissues (Shimasaki *et al*., 2004**).**

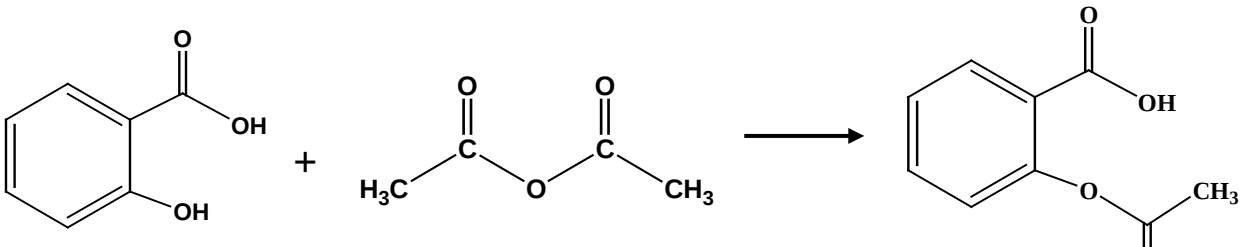
1.2.13.1. Anatomy of Male Reproductive System in Mice

The reproductive system of male mouse consists 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 and bublourethral glands, the testes 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, 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 Leydig cells, which secrete the male sex hormone, testosterone (Mader, 1993).

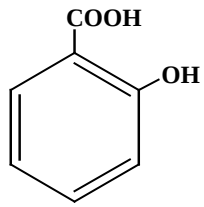
The seminiferous tubules empty into tubular network called the rete testis, which empties into three to five tubules called efferent ductules, these efferent ductules exit the testis into the epididymis, the epididymis is tightly coiled series of thread like tubule located at the posterior side of the 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, the paired seminal vesicles are white, curved, elongated structures notched on the convex surface and hooked at the lateral tips, 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 bublourethral (Cowpers) gland lie lateral to the junction of the membranous urethra and penis (Martini and Mark, 1998).

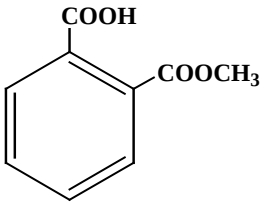


Salicylic acid **Acetic anhydride** **Acetylsalicylic acid (aspirin)**

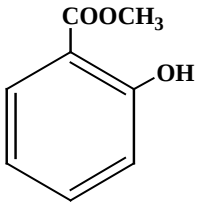
Figure (3): The synthesis of aspirin from salicylic acid (Al-Bayaty, 2002)



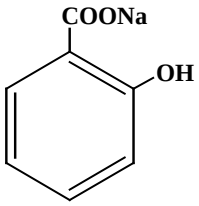
Salicylic acid



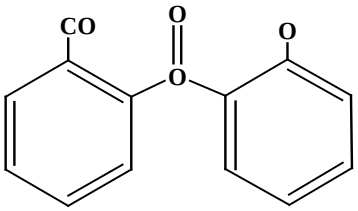
Aspirin



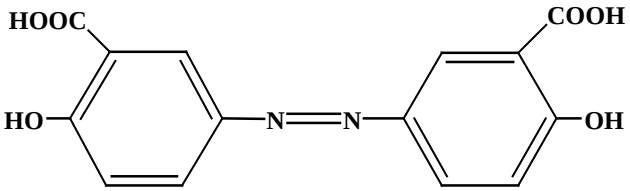
Methyl Salicylate



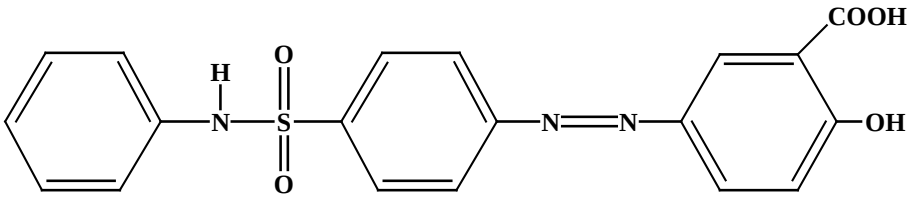
Sodium Salicylate



Salsalate



OLSALAZINE



SULFASALAZINE

Figure (4): Structural formula of the salicylates (Goodman and Gilman's, 1996).

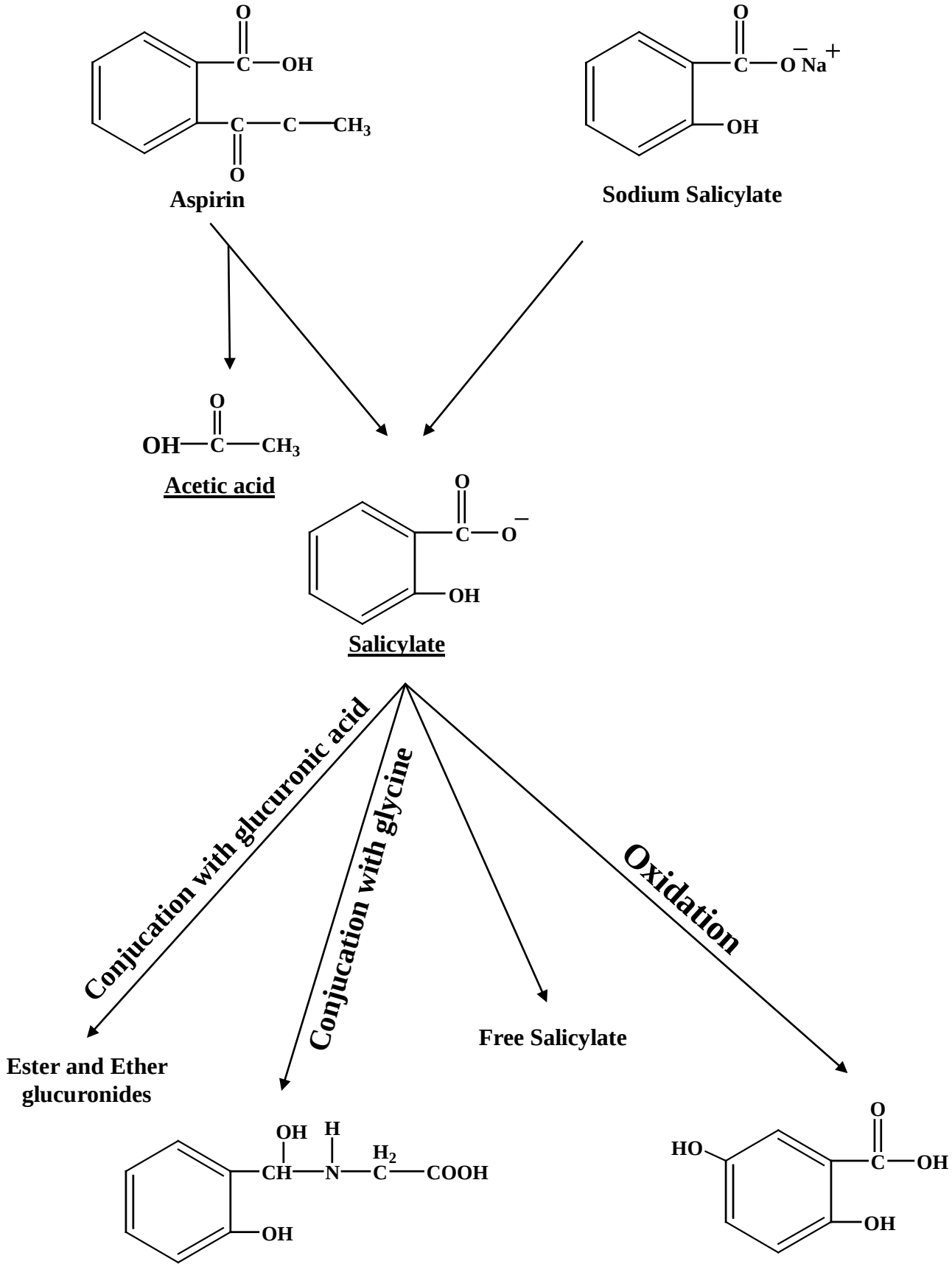


Figure (5): Structural and Metabolism of the Salicylates (Katzung, 2001).

List of Figures

List of Tables

2. Materials and Methods

2.1. Materials

2.1.1. Animals

All experiments were performed on young male Swiss white mice, 5-6 weeks old and with a body weight ranging from 15-20 g. Mice were obtained from the colony of the animal house of the Institute of Embryo Research and Infertility Treatment, Al-Nahrain University. They were kept in air-conditioned room (22-24° C) with an automatically controlled photoperiod (14 hours light and 10 hours darkness). Animals were housed in plastic cages (48 x 15 x 7cm). The bedding material used was fine sawdust which was kept dry and changed every other day. Cages were washed regularly with hot water and disinfected with 70% ethyl alcohol once a week. Mice were fed the standard balanced diet prepared in the animal house of the Institute. Numbering of mice was done using ear or tail marking according to the international system used.

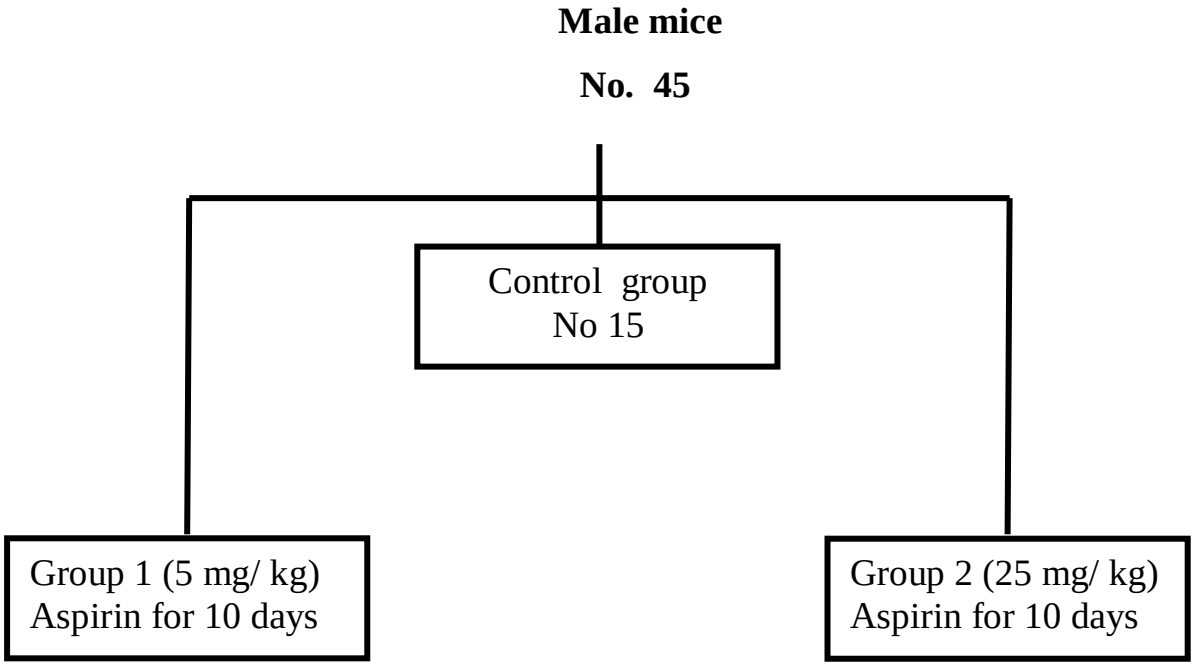


Figure 6: **Experimental design of animal groups.**

2.1.2. Solutions and reagents

2.1.2.1. Chemicals

Table 1: Chemicals used in the experiment.

No.	Chemicals	Company
1	Aspegic® (aspirin)	Laboratories synthelabo, France
2	Chloroform	BDH-England
3	DPX	BDH, Germany
4	Ethanol70%	Ferak, Germany
5	EDTA	BDH, England
6	Eosin	Reidel-DEHaenAG seelze - Hanover, Germany
7	Formalin 40%	BDH, England
8	FSH	CIS biointernational, France
9	Hematoxylin	Ferak, Germany
10	Iodine	Ferak, Germany
11	LH	CIS biointernational, France
12	Testosterone	CIS biointernational, France
13	Wax	BDH, England
14	Xylol	Ferak, Germany

1- Aspirin: It was used in (5 and 25 mg/kg) by diluting 500 mg of Aspegic® (aspirin) powder in 5 ml distilled water followed by decimal serial dilutions and stored in the refrigerator (4 °C until use). The drug was injected interperitoneally within 24 hours.

2- Solutions used for preparation of histological sections:

a- Bouin's solution: It was prepared by dissolving (75 ml) of saturated picric acid with (35 ml) formaline 40% and followed by the addition of (5 ml) of glacial acetic acid, mixed well and left at room temperature for 24 hr. (Bajallan, 2006).

b- Diluting Solution: This solution is composed of 1 ml of 2 % eosin, 1 ml of 3 % sodium chloride and 50 ml of distilled water, it is used for determining of sperm concentration (Zaid and Salhab, 1994).

c- Haemtoxylin -Eosin Solution: (stain)

A- Haemtoxylin stain

B- Aqueous eosin stain was dissolved in 99 ml of D.W.

C- Crystal violet stain: 19 crystals violate powder in 80 ml of D.W. and 20ml of ethanol (95%).

D- LogaL iodine: Ig iodine and 2g (KI) in 300ml D.W.

E-Sodium bicarbonate solution: 0.5mg sodium bicarbonate in 100ml tap water.

F- Acid alcohol: 3drops of concentrated HCL in 200 ml ethanol (0.5%).

G- Giemsa stain solution.

H- Acetic acid solution (0.5%)

1- Xylol.

2.2. Methods

2.2.1. Equipments

Table 2: Experimental Equipments and Company.

No.	Equipments	Company
1	Autoclave	Dixons-UK
2	Centrifuge	Universal-16-A ,Germany
3	Dissecting microscope	Wild, Switzerland
4	Glass-rod	John Poulten Ltd, England
٥	Histokinate	Citadel 1000, Shandon
٦	Light microscope	Olympus ,Japan
٧	Microfuge	Sigma
٨	Refrigerator	Sanyo-Japan
9	Rotary Microtome	Anglia Scientific
١0	Sensitive Balance	Mettler - Switzerland

Those mice were divided into three groups (15 mice/ group) as follows:

1- First group (control) was treated daily with 0.1 ml. of DW. twice/day for 10 days.

a- Animal groups:

2- Second group was experimented with aspirin (5mg/kg BW.) for 10 days in two occasions: the first in 8 am and the second 2 pm and considered as low dose group.

3- Third group was experimented with aspirin (25mg/kg BW.) for 10 days in two occasions: the first in 8 am and the second 2 pm and considered as high dose group.

2.2.2. Body Weight and Testicular 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 (Montanari *et aI.,* 1998). Both testes of each mouse were weighed and expressed in terms of mg/100 g body weight.

2.2.3. Blood collection

Blood was collected from all mice groups (experimental and control) on day 11 after initiation of treatment i.e. 24 hrs after cessation of treatment.

Collection of blood was done by cardiac puncture using 1 ml disposable syringes for insulin injection. The average volume of collected blood was about0.5 ml. Blood was allowed to clot after not more than 15 minutes to be coagulated. Serum was separated using microfuge for 15 minutes at 1500 RPM, obtained sera were frozen in a deep freezer (- 20 ºC) until used for hormonal assy.

2.2.4. Hormonal Assay

The following hormones were assayed using prepared kits Radio-Immunoassay Technique (Al-Rubaee, 2005); the assay was carried out at the central Baghdad laboratory (Ministry of Health).

1. Follicle stimulating hormone (FSH).
2. Luteinizing hormone (LH).
3. Testosterone.

The principle of the assay is based on the competition between the iodine I^{125} labeled hormone and hormone contained in standards or specimens to be assayed for a fixed and limited number of antibody binding sites.

After incubation, the amount of labeled hormone bound to the antibody is inversely related to the amount of unlabeled hormone originally present in the sample. Then the coated tubes washed out as completely as possible, followed by gamma scintillation counter (Hull. *et al*, 1982).

2.2.5. Photography

For stating of results photos have been taken for some histological section using microscope (Olympus compound microscope BH2 and films from the type lucky color GBRI00).

2.2.6. Histological examination

Histological alterations were examined by obtaining some animal tissues such as testes and epididymes, their diameter, thickness, height and interstitial spaces followed by preservation of these organs in preservative solution. The testes and epididymis fixed in Bouin's solution for the preparation of histological sections than 48 hrs and tissues were washed with tap water for few minutes, and left in ethanol (50%) for 30 min., and then were kept in (70%) ethanol that keep the tissues until processing within a week.

Histological technique includes the following steps (Bajallan, 2006):

1. **Fixation**: by Bouin solution.
2. **Washing and dehydration**: samples were washed using (70%) alcohol to remove the remaining Bouin fixative and to avoid any unwanted results, then dehydration used to completely remove water from he tissue by transferring samples into a serial dilutions of ethyl alcohol (80%, 90%, 95% and 100%) and twice for the last two concentrations for (1-2 hrs).

3. **Clearing**: clearing was done to remove of dehydration solution from the samples and then replaced by another solutions that are mixed with paraffin wax, clearing was done by using xylene that was repeated after 0.5-1 hr precede.

4. **Infiltration and Embedding**: samples were incubated in a mixture of clearing solution and paraffin wax at (60ºC) and left for one hour. Embedding was done by using melted paraffin wax for one hour after that, samples were poured in metal containing melted pure paraffin and using hot needle to prevent bubbles formation.

5. **Sectioning**: after solidification of paraffin and before the cutting process removal of unwanted embedding solution around samples which is surrounded by paraffin in thickness (2-3 mm) except the lower surface in thickness (3-5 mm), then samples were cut using rotary microtome to get serial sections with a thickness 7 µm and were put on clean slides smeared from egg albumin, and some drops of distilled water were added in order to spread the tissue. After that sections were

put in hot plate in (45ºC) for the spreading of sections on the surface then drying it.

6. Staining: Haematoxylin -Eosin method (Guyer, 1973) was used.

Histological sections were placed in the following solution and reagents as follow:

a- Xylol for 5 min

b- Absolute alcohol for 1 min

c- Series of graded ethanol (80%, 70%, 50%, and 35%) to be dried then rinsed in distilled water for 1min.

d- Iodine for 1min.

e- Sodium -hypo soleplate to erase the iodine and turn the color of tissue to white and rinsed in tap water for few minutes.

f- Haematoxylin for 1-5 min.

2.3. Statistical analysis

Data analysis is done using Statistical Package for Social Studies (SPSS) and the selection of averages is done according to analysis of ANOVA (F test), then comparison is done in standard errors between the available averages by using DUNCAN test in probability level (0.05) (Duncan, 1955).

Recommendations

1. Further studies are needed to confirm results obtained in this study using other doses for various periods.

2. Other aspects of male physiology should be studied, for example immunological effect, hematological effect.

3. Experiments are needed to be done on other mammals, other than mice.

References

- Abbasi, A. A.; Drinka, P. J.; Mattson, D. E. and Rudman, D. (1993). Low circulating levels of insulin-like growth factors and testosterone in chronically institutionalized elderly men. J. Amer. Geriatr. Soc., 41: 975–982.

- Abou, Y. Z. and Alwan, A. S. (1990). Iraqi Drug Guide. 1st Ed. Aspirin.

- Aitken, J.; Krausz, C. and Buckingham, D. (1994). Relationships between biochemical markers for residual sperm cytoplasm, reactive oxygen species generation, and the presence of leukocytes and precursor germ cells in human sperm suspensions. Mol. Reprod. Dev., 39: 268-279.

- Al-Bayaty, S. A. (2002). Effect of Aspirin on female reproductive system in mice. A thesis submitted to the College of Medicine, University of Baghdad. 1423.

- Al Hasson, D. K. A. (1983). The prostaglandin F2α and the function of the male mice genital system. A thesis submitted to the College of Science, University of Baghdad.

- Al-Rubaee, E. A. (2005). Effect of Alcoholic Extract of Fenugreek Seeds on Fertility in Albino Male Mice. MSc Thesis, University of Baghdad, College of Science for Women, University of Baghdad.

- Alsaady, I. K.; Ajjam, Z.; Abdul-Kareem, H. and Kamal, M. (1989). Reproductive physiology and artificial fertilization. 2nd Ed., Ministry of Higher Education and Scientific Research. Baghdad University.

- Anonomous. (1999). Federal register report of sept., 64: (177): 49655-65.

- Arthur, V.; James, S. and Dorthy, L. (1998). Human Physiology 7th Ed. Mcgraw-Hill companies. inc. for Manufacture and Export. 44(3): 145-148.

- Bajallan, S. (2006). Effect of The Watery Extract of Fenugreek Seeds *(Trigonella foenum-graecum* L.) on Fertility and Some Immuno-parameters in Male Albino Mice. Engl. J. Med., 345: 1–4.

- Bayer, K. (2004). FDA advisory committee underscores need for greater understanding of aspirin's benefit on appropriate patient populations. Engl. J. Med., 7: 102-111.

- Beckman, D. A. and Feuston, M. (2003). Landmarks in the Development of the Female Reproductive System. Birth Defects Research, (part B), 68: 137-143.

- Bhajat, K. (1994). Aspirin. Nursing standard, 8(4): 52-54.

- Bhasin, S.; Storer, T. W.; Berman, N.; Callegari, C.; Clevenger, B.; Phillips, J.; Bunnell, T. J.; Tricker, R.; Shirazi, A. and Casaburi, R. (1996). The effects of supraphysiologic doses of testosterone on muscle size and strength in normal men. Engl. J. Med., 335: 1–7.

- Billard, R.; Cosson, J.; Perchec, G. and Linhart, O. (1995). Biology of sperm and artificial reproduction in carp. Aquacult., 129: 95–112.

- Blanco, F. J.; Guitian, R.; Moreno, J.; De-toro, F. G. and Gald, F. (1999). Effect of anti-inflammatory drugs on cox-2 activity in human articular chondrocytes. J. Rheumatol., 26(6): 1366-73.

- Botting, R. and Vane, J. (1998). Recent considerations in nonsteroidal anti-inflammatory drug gastropathy. Amer. J. Med., 44: 1-10.

- Bowen, J. M. (2004). Prolactin-induced regression of the rat corpus luteum: expression of monocyte chemoattractant protein-1 and invasion of macrophages. Boil. Reprod. 44: 1133-35.

- Brennecke, S. P.; Brwn, M. A.; Crowther, C. A.; Hague, W. M.; King, J.; McCowan, L.; Moris, J.North, R.; Paison, N. and Tippett, C. (1995). Aspirin and prevention of preeclampsia: Position statement of the use of low-dose aspirin in pregnancy by the Australasian society for the study of Hypertension in pregnancy. Aust. N. Z. J. Obst. Gynaecol., 35(1): 35-41.

- Carey, F. A. (1987). *In vitro* fertilization: determination of follicular maturation for timing of human chorionic gonadotrophin administration. Fertil. Steril., 47: 345-349.

- Cerletti, C.; Dell'Elba, G.; Manarini, S.; Pecce, R.; Castelnuovo, A.; Scorpiglione, N.; Feliziani, V. and gaetano, G. (2003). Pharmacokinetic

and pharmacolodynamic differences between low-dosage of aspirin may affect therapeutic outcomes. Biol Reprod., 78: 119- 124.

- Chandra, J. and Bhatnagar, S. K. (2002). Antipyretics in children. India. J. Pediatr. 69(1): 69-74.

- Cuthbertson, L. L.; Lastner, U.; Glasl, S.; Saukel, J. and Jurenitsch, J. (1981). Anti-inflammatory agents and other drugs and its relationships with sperm formation in rat. Fertil. Steril., 41(2): 236-243.

- Cuthbertson, L. L. and Cobbold, T. (1985). The endocrine regulation of spermatogenesis: Independent role for testosterone and FSH. J. Endocrinol., 148:1-9.

- Dejucq, N. and Jégou, B. (1998). Viruses in the Mammalian Male Genital Tract and Their Effects on the Reproductive System, Biol Reprod., 32: 1132- 44.

- Dierich, A.; Sairam M. R.; Monaco, L.; Fimia, G. M.; Gansmuller, A.; LeMeur, M. and Corsi, P. S. (1998**).** Impairing follicle-stimulating hormone (FSH) signaling *in vivo*: Targeted disruption of the FSH receptor leads to aberrant gametogenesis and hormonal imbalance. Cell Biology, 95 (23): 13612-17.

- Duncan, D. (1955). Multiple ranges and Multiple F-tests. Biometrics. 11: (1-42).

- Dym, M. and Fawcett, D. W. (1970). The Blood-Testis Barrier in the Rat and the Physiological Compartmentation of the Seminiferous Epithelium. In: Biol Reprod., 3: 308-326.

- Ebodi, M. (1996). Pharmacology in illustrated review with questions and explanations, 3rd Ed., Mechanisms of action of aspirin. 4: 105.

- Ferrando, A. A.; Sheffiled-Moore, M. A.; Yeckel, C. W.; Gilkison, C.; Jiang, J.; Achacosa, A.; Lieberman, S. A.; Tipton, K.; Wolfe, R. R.; and Urban, R. J. (2001). Testosterone administration to older men improves muscle function: molecular and physiological mechanisms, British J. Gasroenterol., 11(7): 56-63.

- Ferrira S. H.; Moncada S. and Vane J. (1971). Indomethacin and aspirin abolish prostaglandin release from the spleen, Nature. 231: 237-239.

- Flower, R. J. (2006). Prostaglandins, bioassay and inflammation. British J. Pharmacol., 147 (1): 182–92.

- Foster, L. H.; Small, J. D. and Fox, G. F. (1982). Yhe mouse in biomedical research. Public press, INC. (London). 123(3): 134-145.

- Frank, W. and Jackson, M. D. (1998). Aspirin, hormones and others. British J. Gasroenterol., 56(4): 34-37.

- Funk, C. D.; Funk, L. B.; Kennedy, M. E.; Pony, A. S. and Fitzgerald, C. A. (1991). Human platelet / erythro-Leukemia chromosomal assignment. FASEB J., 2304-12.

- Gage, M. J. G.; Stockley, P. and Parker, G. A. (1995). Effects of alternative male mating strategies on characteristics of sperm

production in the Atlantic salmon (Salmo salar): theoretical and empirical investigations. Phil.Trans. R. Soc. Lond B., 350: 391–399.

- Ganong, W. F. (2003). Review of Medical Physiology. A Lange medical book, 21 Ed., McGraw-Hill. 121: 24-30.

- Garella, S. (1988). Extracorporeal teqniques in the treatment of exogenous intoxications. J. Kidney int. 33: 734-735.

- Garland, H.O.; Phipps, D.J. and Harpur, E.S. (1992). Non steroidal induced hypercalciuria in the rat: assessment of nephron site involved. J. Pharmocol. Exp. Ther., 263(1): 293-297.

- Goldstein, A. L.; Guha, A.; Zatz, M. M.; Hardy, M. A. and White, A. (1972). Purification and Biological Activity of Thymosin, a Hormone of the Thymus Gland. Proc. Natl. Acad. Sci. U S A., 69(7): 1800–1803.

- Gomendio, M. and Roldan, E. R. S. (1991). Sperm size and sperm competition in mammals. Proc. R. Soc. Lond B., 243: 181–185.

- Goodman, R. and Gilman, S. (1996). Analgesic-Antipyretic and Anti-inflammatory-agents. The pharmacological basis of therapeutics 9th Ed. Mc Graw Hill., New York.

- Gordon, I. J. (1984). Algorith for modified alkaline duuresis in salicylate poisoning. British med. J., 289: 1039-44.

- Guyer, M. (1973). Animal microbiology 5th Ed. The University of Chicago Press. Chicago. 365(3): 165-172.

- Ghosh, S. and Dasgupta, S. (1998). Non steroidal drugs induced inhihibition of steroidogenic enzymes in rat testis. Indian J. Physiol. Pharmacol., 41(2): 247-50.

- Harder, A. T. and Yuehuei, H. **(**2000**).** The Mechanisms of the Inhibitory Effects of Nonsteroidal Anti-Inflammatory Drugs on Bone Healing, Amer. J. Physiol. Pharmacol., 9: 188-198.

- Harrison's, T. R. (2001). Principles of internal medicine. 15th Ed. McGraw-Hill. U.S.A.

- Henry, J. and Volans, G. (1984). ABC of poisoning. Analgesic poisoning-1. British Med. J. 289: 820-825.

- Higgs, G. A.; Salmon, J. A.; Henderson, B. and Vane, J. R. (1999). Pharmacokinetics of aspirin and salicylate in relation to inhibition of arachidonate cyclooxygenase and antiinflammatory activity. Indian J. Physiol. Pharmacol., 11: 223-227.

- Hirsh, J.; Dalen, J. E.; Fuster, V.; Harker, L. B. and Salzman, E. W. (1992). Aspirin and other platelet-active drugs: the relationship between dose, effectiveness and side effects. Chest. 102(1): 327-336.

- Horton, E. W.; Jones, R. L. and Marr, C. G. (1991). Effect of aspirin on prostaglandin and Fructose Levels in human semen. J. Reprod. Fert., 33: 385-392.

- Hsiech, Y. Y.; Tsai, H. D.; Chang, C.; Lo, H. Y. and Chen, C. L. (2000). Low-dose aspirin for infertile women with thin endometrium receiving intrauterine insemination in a prospective, randomized study. J. Assist. Reprod. Cent. 17(3): 137- 142.

- Hull, M. G. R.; Savage, P. E. and Akashi, H. X. (1982). The value of single serum progesterone measurement in the midluteal phase as a criterion of a potentially fertile cycle (ovulation) derived from treated and untreated conception cycles. Fertil-Steril. 37: 325-327.

- Hurst, B. S.; Bhojwani, J. T.; Marshburn, P.B.; Papadakis, M. A. Loeb, T. A. and Matthews, M. L. (2002). Low-dose aspirin does not improve ovarian stimulation, endometrial response, or pregnancy rates for *in vitro* fertilization, Amer. J. Med., 7: 110-122

- Huszar, G.; Ozenci, C. C.; Cayli, S.; Kovacs, T.; Vigue, L. and Kovanci, E. (2004). Semen Characteristics after Overnight Shipping: Preservation of Sperm Concentrations, HspA2 Ratios, CK Activity, Cytoplasmic Retention, Chromatin Maturity, DNA Integrity, and Sperm Shape. J. Androl., 25(4): 48-55.

- Igusa, H. Y. and Miyazaki Y. I. (1986). Effect of aspirin in surgery of testes is dependent on increasing of bleeding time. Amer. J. Gynaecol. 180(2): 142-146.

- Irvine, D. S.; Allan, I. W.; Macnamee, M. and Aitken, R. J. (1997). Field trial of a diluent for the transportation of human semen at ambient temperatures. Fertil. Steril., 67: 348–354.

- Jackson, M. D. (2005). Aspirin and its correlations with hormones and immediators. A preliminary study. J. Amer. Geriatr. Soc., 45: 123–125.

- Jacob, L. (1996). NMS pharmacology. 4th Ed. Aspirin. Wiliams and Willkins, Non steroidal anti-inflammatory drugs. P: 195.

- Jones, K. T.; Carrol, J.; Merriman, J. A.; Whittingham, D. G. and Kono, T. (2001). Repetitive sperm-induced Ca2+ transients in mouse oocytes are cell cycle dependent. Terrace, London, SW17 0RE, UK. 133: 32-36.

- Jones, R. (2001). Nonsteroidal anti-inflammatory drug prescribing: past, present and future. Amer. J. Med., 110(1): 45-52.

- Kalthoff, K. (2001). Analysis of biological development. 2nd Ed. McGraw company New York.

- Katzung, B. G. and Furst, D. E. (2001). Basic and clinical pharmacology 8th Ed. Nonsteroidal anti-inflammatory drugs. Amer. J. Gynaecol., 21: 65-77.

- Kline M. G. and Kline, R. (1992). The endocrine regulation of spermatogenesis: Independent role for testosterone and FSH. J. Endocrinol., 148:1-9.

- Khan, T.; Bosch, J.; Wiener, P. and Dikmon, S. (1980). Course of gentamicin nephrotoxicity. Toxicology, 16:49-57.

- Krause, E.; Bohring, C.; Habermann, B. and Krause, W. (2001). Isolation and identification of sperm membrane antigens recognized by antisperm antibodies, and their possible role in immunological infertility disease. Mol. Hum. Reprod., 7: 113-118.
- Laurence, D. R. (1997). Clinical pharmacology. 8th Ed. Inflammation, arthritis and none steroidal anti-inflammatory drugs. Churchill living stone U.S.A. 432: 12-17.
- Leach, D. H. (1997). Studies on the effect of some antihistamines on the reproductive organs of male rates and its advantage in sperm competition. Vet. Med. J., 31(2): 277-286.
- Lee, M.; Cryer, B. and Feldman, M. (1994). Dose effect of aspirin on Gastric prostaglandins and gut mucosal injury. Mol. Hum. Reprod., 120(3): 184-189.
- Lenz, T and Wislon, A. (2003). Clinical pharmacokinetics of the antiplatelet agents used in the secondary prevention of stroke. Clin. Pharmacokinet. 42(10): 909-20.

- Levesque, H. and Lafout, O. (2000). Aspirin through the ages: a historical review. Rev. Med. Intern. 21 Suppl. 1: 8-17.

- Li, K. M.; Kanamasa, K. and Ogawa, I. (2001). Aspirin (50 mg) per day combined with either dipyridamol or ticlopidine is effective to prevent recurrent myocardial infraction. Circulation. 86(1): 641-643.

- Lin, T.; Calkim, J. and Morris, P. (1989). Regulation of leyding cell function in primary culture by inhibin and activin. Endocrinology, 125: 2134-40.

- Lippincott, S. (2000). Illustraed reviews: Phamacology 3rd Ed. Anti-inflammatory drugs. New York. 2441 (2): 233-245.

- Loll, P. J.; Picot, D. and Caravito, R. M. (1995). The structural basis of aspirin activity inferred from the crystal structure of inactivated prostaglandin H_2 synthase. Nat. Struct. Biol. 2(8): 637-43.

- Lorenz, R. L.; Boehling, B.; Vederhoven, W. M. and Weber, P. C. (1989). Superior antiplatelet action of alternative day pulsed dosing versus split dose administration of aspirin. Amer. J. Cardiol., 64: 1186-88.

- Mader, T. A. (1993). Low-dose aspirin improves testicular responsiveness, spermatogenesis and capacity of semeniferous fluid in male, implantation and pregnancy rate in patients undergoing IVF: a prospective, randomized, double-blind placebo-controlled assay, Fertil. Steril., 15(4): 815-819.

- Makino, T.; Demers, L. M. and Freep, R. O. (1974). Factors influencing pituitary glycogen metabolism and Gonadotropic hormone release. II. A role of prostoglandins. Fertil. Steril. 883-885.

- Manicardi, T. L.; Abbott, M.; Lair, D. N. C.; McNeilly, A. S.; Nestor, J. J.; Vickory, B. H. and Fraser, H. M. (1995). Effect of an LH releasing hormone antagonist on the secretion of LH, FSH, Testosterone at different stages of spermatogenesis in albino rabbit. J. Endocrin. 112: 67-74.

- Martini, F. H. and Mark, D. (1998). Fundamentals of Anatomy and Physiology. Prentice-Hall, New Jersey. Endocrinology, 125: 2134-40.

- Meade, E. A.; Smith, W. L. and Dewit, D. L. (1993). Differential inhibition of prostaglandin endoperoxide synthase, cyclogenease isozymes by aspirin and other non steroidal anti-inflammatory drugs. J. Biol. Chem., 268: 6610-14.

- Montanari, T.; De Carvalho, J. E. and Dolder, H. (1998). Antispermatogenic effect of *Achillea millefolium* L. in mice contraception, 58(5): 309-313.

- Morley, J. E.; Perry, H. M.; Kaiser, F. E.; Kraenzle, D.; Jensen, J.; Houston, K.; Mattammal, M. and Perry, H. M. (1993).Effects of testosterone replacement therapy in old hypogonadal males: a preliminary study. J. Amer. Geriatr. Soc., 41: 149–152.

- Ng, C. M.; Blackman, M. R.; Wang, C. and Swerdl, R. S. (2004). The Role of Carnitine in the Male Reproductive System. J. Endocrin., 1033: 187-188.

- Parker, G. A. (1993). Sperm competition games: sperm size and sperm number under adult control. Proc. R. Soc.Lond., 253: 245–254.

- Patrignani, P.; Panara, M. R.; Greco, A.; Fusco, O.; Natoli, C.; Iacobelli, S. C.; Pollone, F.; Ganal, A.; Creminone, C. and Maclouf, F. J. (1995). Characterization of the cyclooxygenase activity of human blood prostaglandin endoperoxid synthesis. Adv. Prostaglandin. Thrombox Leukot. Res. 23: 129-133.

- Patrono, C. (2001). Aspirin: new cardiovascular uses for an old drug. Amer. J. Med., 110: 62-65.

- Porter, D. R. and Sturrock, R. D. (1993). Medical management of Rheumatoid arthritis. British Med. J., 331: 1711-13.

- Range, H. P.; Pale, M. M. and Ritter, J. M. (1999). Pharmacology, 4th Ed. Salicylate. 32: 235-236.

- Raven, P. H. and Johanson, G. B. (1996). Biology. 14th Ed. McGraw-Hall Company. USA. 12: 43-55.

- Rumble, D. T.; hanssen, R. G. and Kloosterboer, H. J. (1980). Development and characterization of an in vitro spermatogenesis model using rat semeniferous tubules. Biol. Reprod. 54(2): 402-404.

- Rice, C. A. and. Pollard, J. M. (2004). Aspirin beyond Pain Relief. Extension Associate-Health, 8 (8): 117-123.

- Sakkas, R. F.; Lin, T.; Calkim, J. and Morris, P. (1999). Regulation of leydig cell function in primary culture by inhibin and activin. Endocrinolology. 125: 2134-40.

- Saladin, K. S. and Porth, C. M. (1998). Anatomy and Physiology, the unity of form and Function. McGraw-Hill, USA. Amer. J. Med., 5 (6)**:** 245-257.

- Schafer, A. J. (1999). Effects of nonsteroidal anti-inflammatory therapy on platelet. Amer. J. Med. 31; 106(5): 25-36.

- Seeley, R. R.; Trent, D. S. and Philip, D. A. (1996). Anatomy Physiology 2nd Ed. McGraw-Hill companies. Inc. for Manufacture and Export. 23(3): 176-182.
- Shimasaki, S.; Moore, R. K.; Otsuka, F. and Erickson, G. F. (1993). The Bone Morphogenetic Protein System in Mammalian Reproduction. Amer. J. Med., 8 (7)**:** 255-267.
- Sneader, W. (2000). The discovery of aspirin. B. M. J., 321(7276): 159-164.

- Square, D. (1997). Amyloid beta protein and Alzheimer's disease. C. M. A. J., 156: 1040-42.

- Stillings, M.; Havlik, I.; Chetty, M.; Clinton, C.; schall, R.; Modley, I.; Muir, N. and Littles, S. (2000). Comparison of pharmacokinentic profiles of soluble aspirin and solid paracetmol tables in fed and fasted volunteers. Curr. Med. Res. Opin., 16(2): 115-124.
- Taylor, G. S.; Tramura, K. and Kogo, H. (1993). The mode of action of indomethacin, aspirin and melatonin on the blockage of the first spermatogenesis in immature rat pretreated with PMSG. J. Pharmacol. 50 (3): 467-480.
- Treier, M.; Connell, S. O.; Gleiberman, A.; Price, J.; Szeto, D. P.; Burgess, R.; Chuang, P.; McMahon, A. P. and Michael, G. (2001). Hedgehog signaling is required for pituitary gland development. 34: 1956-74.
- Trudinger, B. J.; Cook, C. M. and Thrompson, R. S. (1988). Low-dose aspirin therapy improves fetal weight in umbilical placental insufficiency. Amer. J. Obstet. Gynaecol., 159: 681-683.
- Vane, J. R. and Botting, R. M. (2003). The mechanism of action of aspirin. Thromb. Res., 110(5): 255-258.
- Vane, J. R.; Bakhle, Y. S. and Botting R. M. (1998). Cyclo-oxygenase-1 and 2. Ann. Rev. Pharmacol. Toxicol., 38: 97-118.
- Verbeeck, P. K. (1990). Pharmacokinetic drug interactions with nonsteroidal anti-inflammatory drugs. Clin. Pharmacokinet., 19: 44-57.

- Wallenberg, H. C.; Rotman, S. N. (1987). Prevention of recurrent idiopathic fetal growth retardation by low-dose aspirin and dipyridamole. Amer. J. Obstet. Gynaecol. 157: 1230-35.

- Wang, S.; Swerdloff, R. S.; Iranmanesh, A.; Dobs, A.; Snyder,P. J.; Cunningham, G.; Matsumoto, A. M.; Weber, T. and Berman, N. (2000). Transdermal Testosterone Gel Improves Sexual Function, Mood, Muscle Strength, and Body Composition Parameters in Hypogonadal Men. J. Clin. Endocrinol. Metab., 85(8): 2839-53.

- Ward, D.; Celik-Ozenci, C.; Catalanotti, J.; Jakab, A.; Aksu, C.; Bray-Ward, P.; Demir, R. and Huszar, G. (2003). Human sperm maintain their shape following decondensation and denaturation for fluorescent in situ hybridization: shape analysis and objective morphometry. Biol. Reprod., 69: 1347–55.

- weber, A. A.; Przytulski, B.; Schanz, A. Hohlfed, T. and Schror, K. (2002). Towards a definition of aspirin resistance a typological approach-platelets. 13(1): 37-40

- Williams, K. (1995). Hypertension in pregnancy. Can. Fam. Physician., 41: 626-632.

- Xu, X. M.; Sansores-Garcia, L.; Chen, X. M.; Matijevic, A. N.; Du, M. and Wu, K. K. (1999). Proc. Natl. Acad. Sci., 96: 5292-97.

- Yen-s, S. C.; Jaffe, R. B. and Barbieri, R. L. (1999). Reproductive endocrinology. Physiology, pathophysiology and clinical management. 4th Ed. W.B. Saunders Company. Biol. Reprod., 54: 1347–55.

- Zaid, A. A. and Salhab, S. A. (1994). Reoroductive physiology and *In vitro* fertilization (practical part). 1st. Ed. Umar Al-Mukar University. Libya. Pharmacol. Toxicol., 38: 97-118.

- Zangwill, M. (1998). The Benefits and Risks of Aspirin and its relationship with many of body systems. Biol. Reprod., 65(5): 1023-30.

3. Results and Discussion

3.1. Changes in Body Weight

Concerning changes in body weight although decrease in the body weight was observed in aspirin treated mice as compared with control animals, the decrease was non significant in experimental group 1 as compared to control, while it was significant (P<0.05) in experimental group 2 when compared with experimental group 1 and control as shown in (Figure 7), when the mice were treated with (5mg/kg and 25mg/kg) of body weight, similar results were obtained by (khan, 1980; Garland *et al.*, 1992). The reason for that is due to the loss of appetite caused by continuous administration of the drug (Garland *et al.*, 1992).

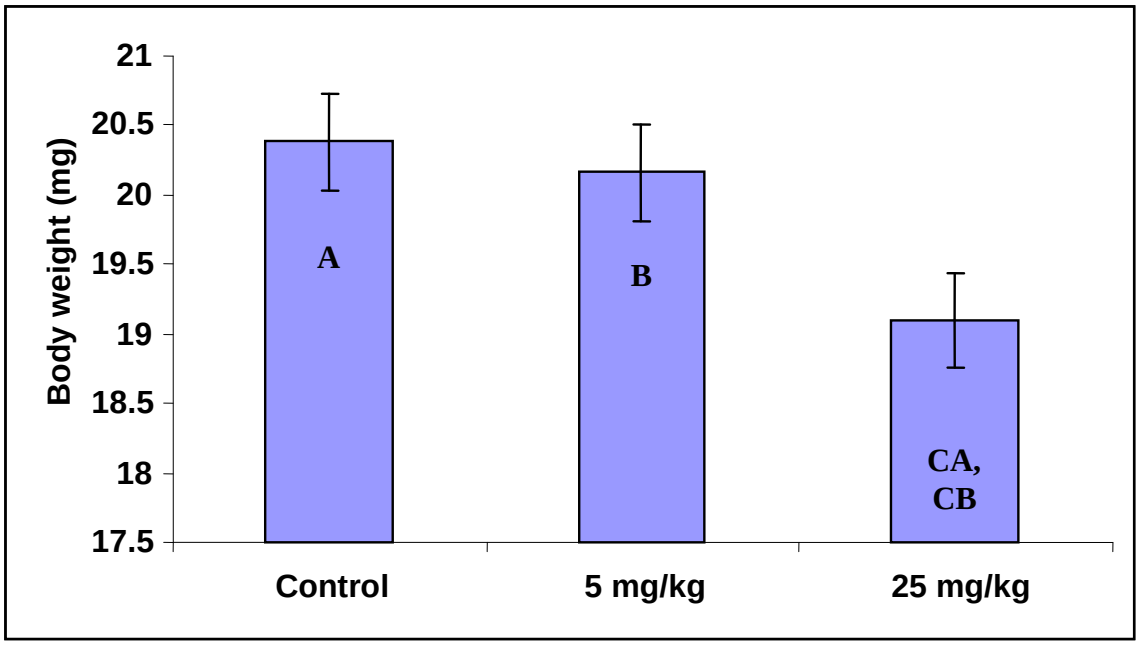


Figure 7: Changes in body weights associated with aspirin administration (5mg/kg) and (25mg/kg) for 10 days twice a day.

* Two letters = significant difference (p<0.05).

3.2. Changes in Testicular and Epididymal Weights

A highly significant decrease ($P < 0.001$) in both testicular and epididymal weights was observed following aspirin treatment (5 and 25 mg/kg) twice daily when compared with the control (Figures 8 and 9).

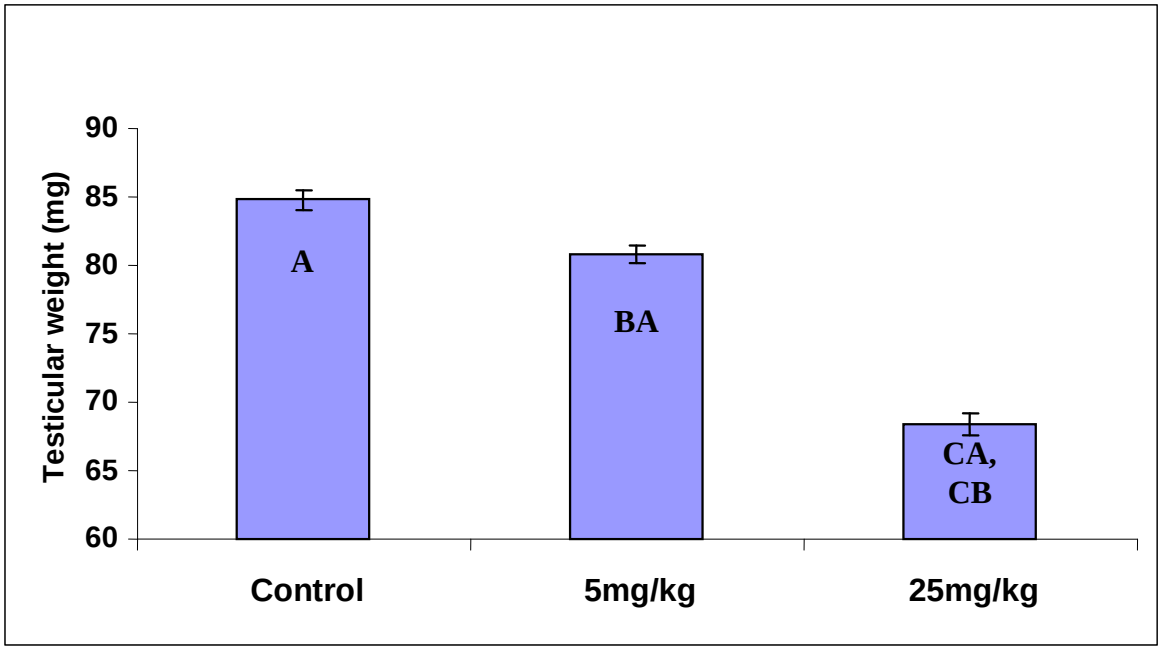


Figure 8: Changes in testicular weights associated with aspirin administration at (5mg/kg) and (25mg/kg) for 10 days twice a day.

* Two letters = significant difference ($p<0.001$).

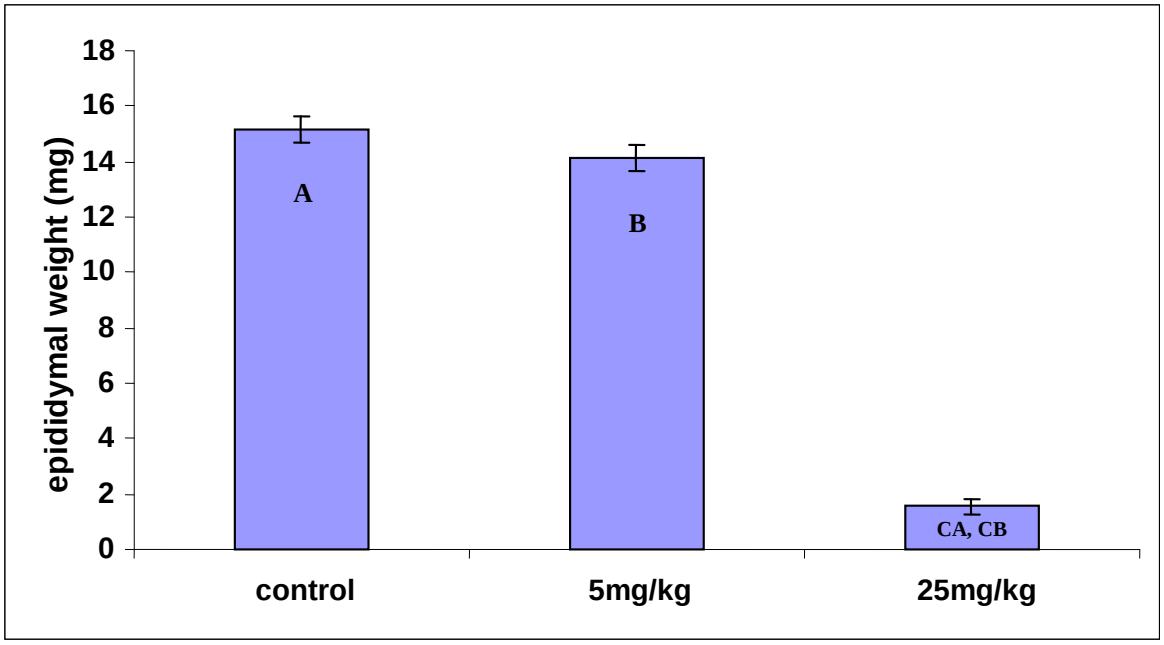


Figure 9: Changes in Epididymal weights associated with aspirin administration at (5mg/kg) and (25mg/kg) for 10 days twice a day.

* Two letters = highly significant decrease ($p<0.001$).

These changes indicate that, aspirin at the injected doses must have disturbed mechanism(s) that control testicular and epididymal weights. It is well known that both FSH and LH are the hormones control testicular growth and weight (Alsaady *et al*., 1989). While epididymal weight and growth is controlled in addition to these hormones by testosterone secreted by testes (Harrison, 2001). In other words, any damage to the testes (main source of testosterone) will affect adversely epididymal growth. Tracing available literature, through various ways of modern literature review showed that very little work has been done on this area. Ghosh and Dasgupta (1999), for instance have shown that aspirin cause disruption of spermatogonia. For aspirin effect, few papers have shown**,** however that in the female, aspirin causes ovarian weight decrease (Al-Rubaee, 2005).

From these figures, observed a marked decrease in the rate of testes weights and epididymis weights after the killing period (after 10 days) of the animals.

3.3. Testicular and epididymal structural changes

The structural changes in the testes and epididymis obtained in the experimental animals are shown in figures (9, 10, and 11). In the testes, the seminiferous tubular diameter showed non significant decrease in both experimental groups as shown in (Figure. 10).

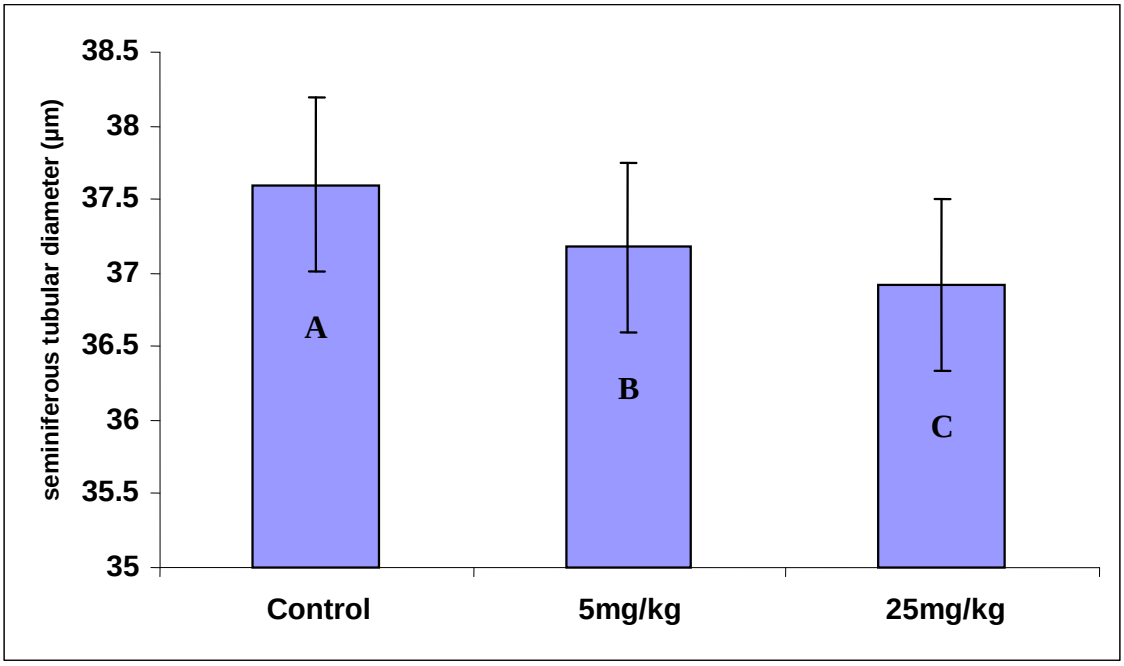


Figure 10: Changes in Seminiferous tubular diameter associated with aspirin administration at (5mg/kg) and (25mg/kg) for 10 days twice a day.

* One letter = non significant difference.

But about to the thickness of spermatocyte cells of the seminiferous tubules a significant decrease ($P < 0.05$) in experimental group 2 as compared with control group. Also there was a slightly decrease in experimental group 1 as compared to control but in significant (Figure. 11).

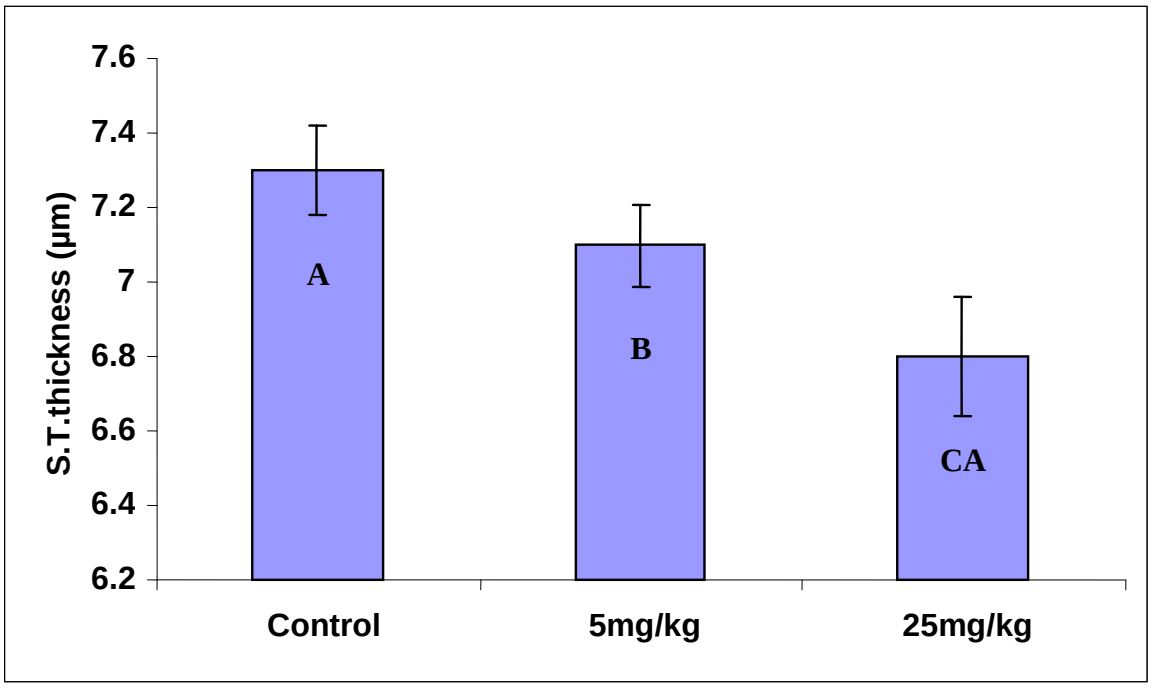


Figure 11: Changes in seminifrous tubular thickness associated with aspirin administration at (5mg/kg) and (25mg/kg) for 10 days twice a day.

* Two letters = significant difference ($p<0.05$).

Also there was a significant increase ($P < 0.05$) in the spaces between seminiferous tubules in experimental group 2 as compared to control group (Figure. 12).

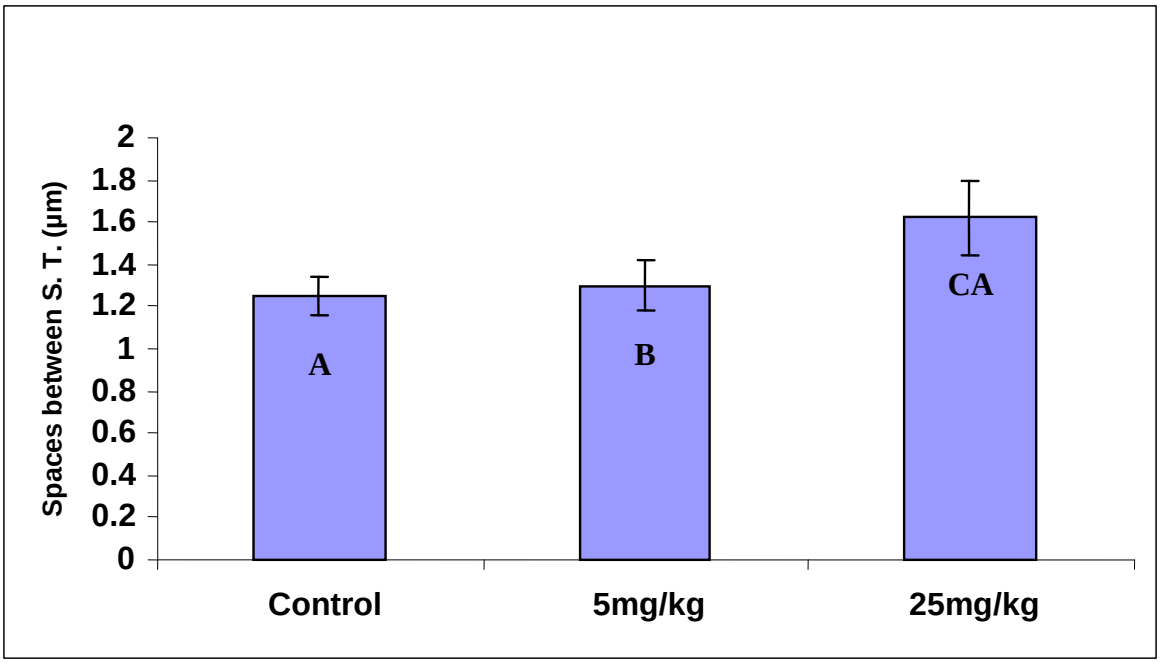


Figure 12: Changes in spaces between seminiferous tubules associated with aspirin administration at (5mg/kg) and (25mg/kg) for 10 days twice a day.

* Two letters = significant increase ($p<0.05$).

While in the epididymis a highly significant decrease ($P < 0.001$) in epididymal cells diameter in both experimental groups as compared with control, and between the two experimental groups (Figure. 13).

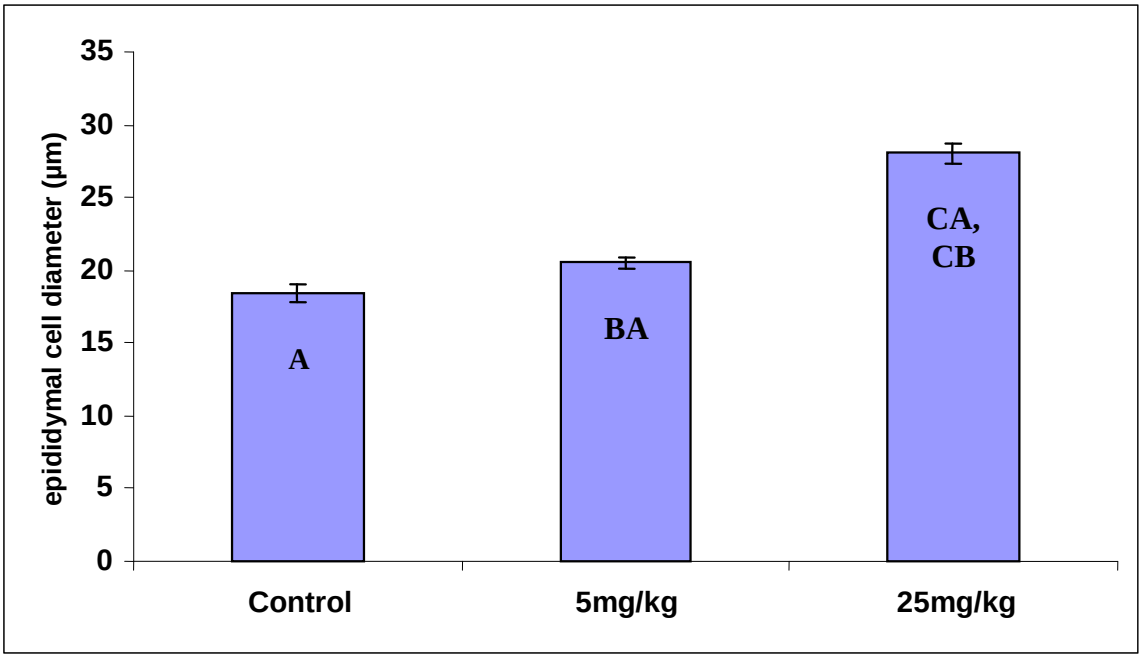


Figure 13: Changes in epididymal cell diameter associated with aspirin administration at (5mg/kg/day) and (25mg/kg/day) for 10 days twice a day.

* Two letters = highly significant increase ($p<0.001$).

Also there was a highly significant decrease ($P < 0.001$) in epididymal cells height in experimental group 2 as compared with control and experimental group 1 (Fig. 14).

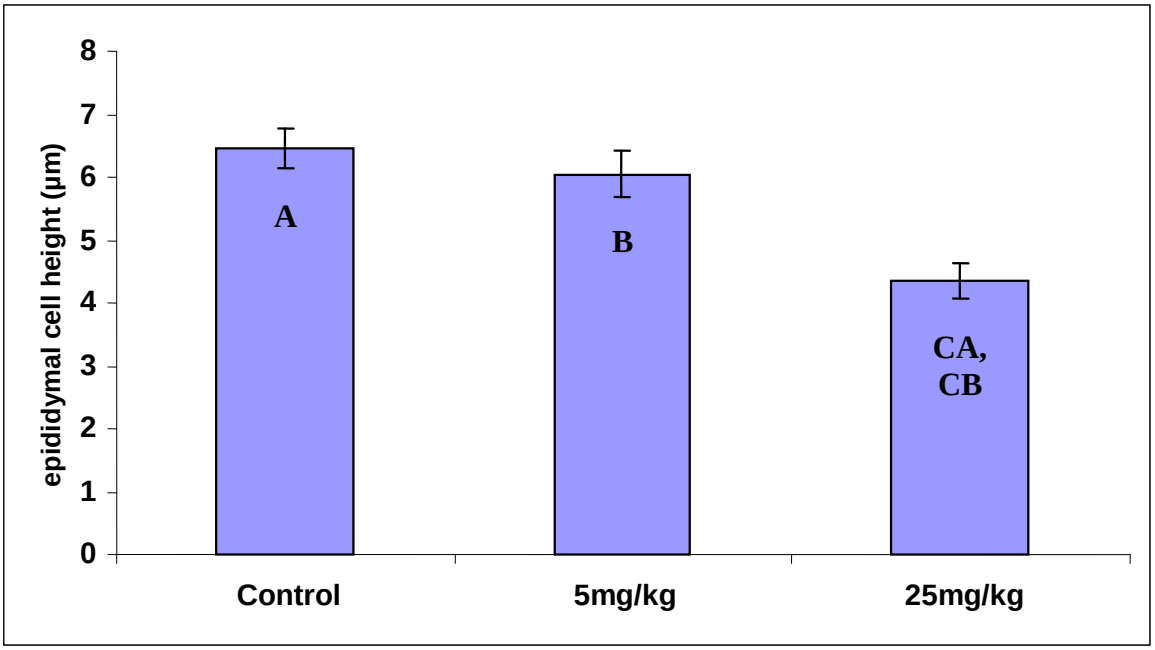


Figure 14: Changes in epididymal cell height associated with aspirin administration at (5mg/kg/day) and (25mg/kg/day) for 10 days twice a day.

* Two letters = highly significant decrease ($p<0.001$).

The structural changes in the testes and epididymis may have been brought about either by a direct action of aspirin on these organs or centrally on their hormonal regulation. Since aspirin is known to inhibit PGs synthesis in various tissues including the reproductive system (Horton *et al.*, 1991) and PGs are needed for normal function of reproductive organs (Lee *et al.*, 1994). Aspirin inhibits the enzyme COX (PGG/H synthase) (Ebodi, 1996). COX is the enzyme that catalyses the conversion of archidonic acid endoperoxide compounds, and hence inhibits formation of PGs. So it is not surprising by the changes obtained in the present study. Central effect of aspirin has been known for years (Meade *et al.*, 1993).

Aspirin has been found to induce suppressions of hypothalamic PGE_2 (Chandra and Bhatnagar, 2002), and thus preventing decreasing gonadotropic tropic pituitary hormones and thus decreasing hormonal support for the testes and decreasing its capacity for the synthesis of testosterone. The significant decrease in the diameter and thickness of the seminiferous tubular diameter and thickness of their wall with increasing the inter seminiferous tubular diameter coupled with absence a decrease in the interstitial cells. These cells are known to produce testosterone induce the influence of LH (Jackson, 2005). Decrease LH means decreasing testosterone biosynthesis this will affect not only the testes but also the epididymis (Ganong, 2003). These changes seen in the epididymis are consequence of decreasing testosterone.

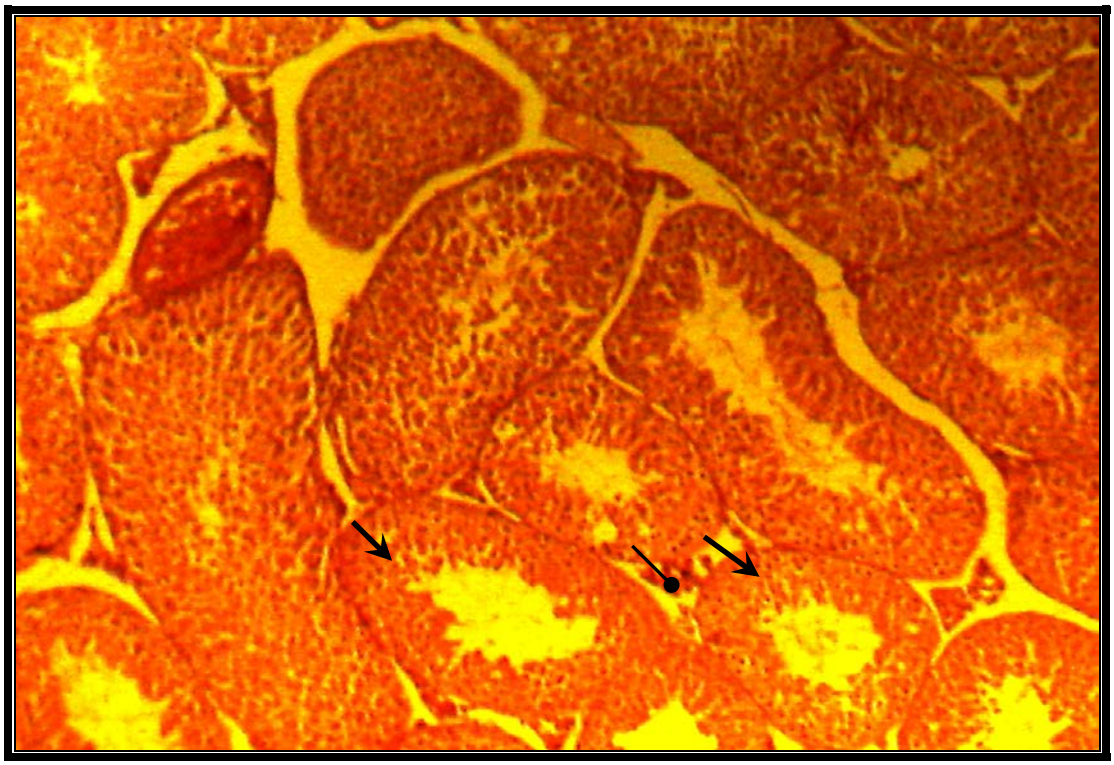


Figure 15: A histological picture of the testis shows the seminiferous tubules of the control group. (Haematoxylin and Eosin X400).

- ↘ : This arrow indicates spermatogenic cells**.**

- ↘ : This arrow indicates blood vessels.

Sections of the testes reveled presence of seminiferous tubules with some not clearly recognized changes with multi rows of spermatogenic cells, their lumen again appear to be empty. Interstitial spaces contain few blood vessels and very few interstitial cells (Figure. 15).

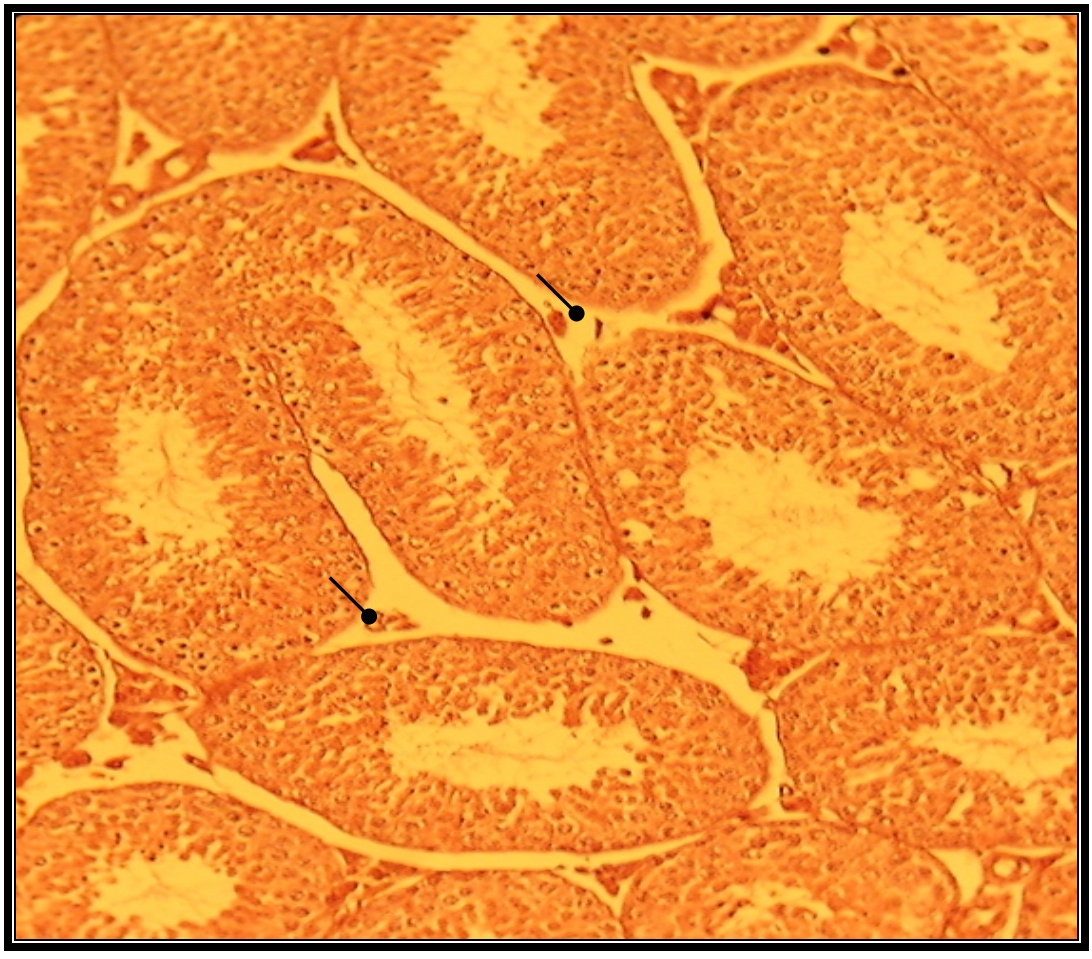


Figure 16: A histological picture of the testis treated with (5 mg/kg of b.w.) aspirin shows the seminiferous tubules of the experimental group 1. (Haematoxylin and Eosin X400).

- ↘ : This arrow indicates blood vessels.

Sections of the testes reveled presence of seminiferous tubules with multi rows of spermatogenic cells, their lumen appear to be empty. Interstitial spaces contain blood vessels and very few interstitial cells (Figure. 16).

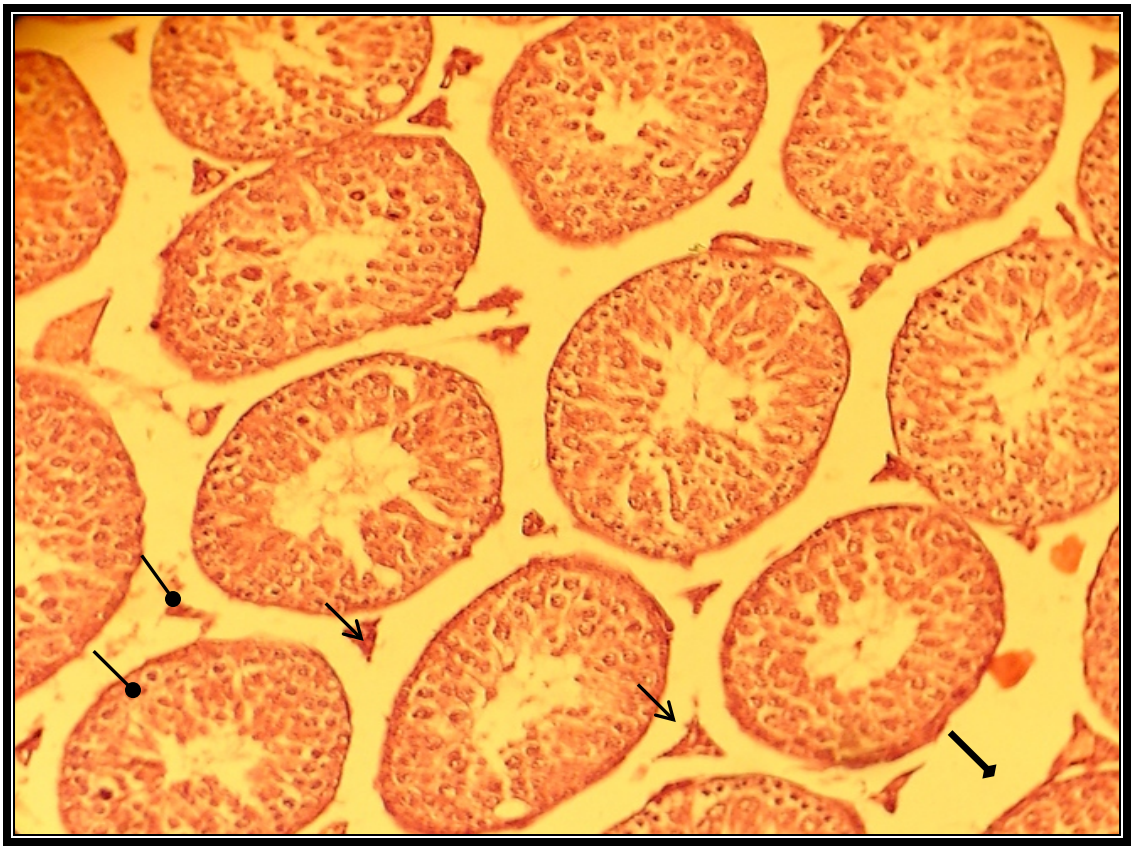


Figure 17: A histological picture of the testis treated with (25 mg/kg of b.w.) aspirin shows the seminiferous tubules of the experimental group 2 with wider interstitial spaces. (Haematoxylin and Eosin X400).

- ↘ : This arrow indicates interstitial spaces.
- ↘ : This arrow indicates blood vessels.
- ↘ : This arrow indicates interstitial cells.

Testicular changes in experimental group 2 included degenerative changes in the seminiferous tubules as shown by the presence of wide almost empty spaces between the sections of tubules, their diameter (longitudinal and

transverse was less than the control and first experimental group, although it was insignificant (Figure 17). The lining of the seminiferous tubule contains empty spaces, cells appear to be loose and their lumens are wide and appear to be empty. Interstitial spaces are wide and very few blood vessels are found with scattered very few interstitial cells.

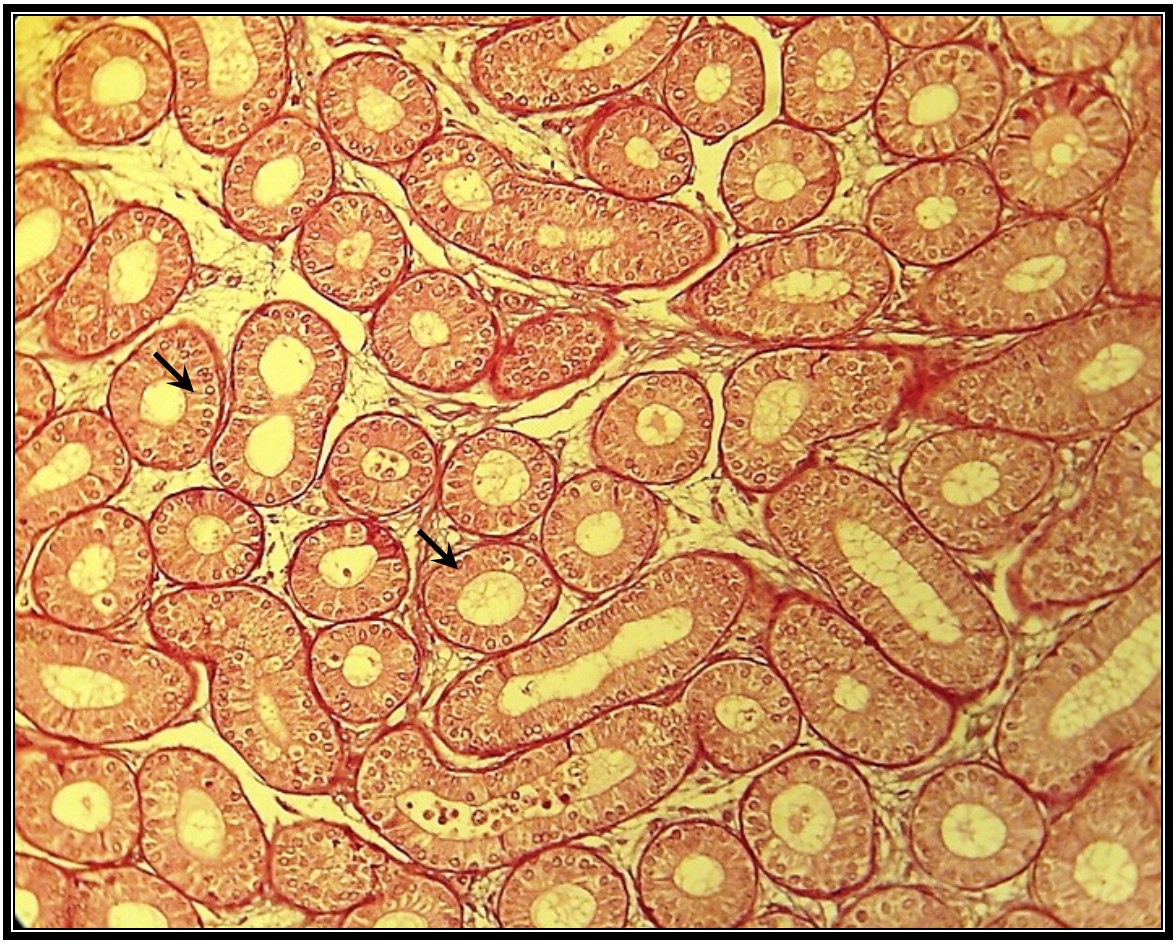


Figure 18: A histological picture of the epididymis shows the tubules of the control group. (Haematoxylin and Eosin X100).

- ↘ : This arrow indicates lining cells.

Sections of tubules appear to be compact with small spaces between them and the tubules are showed cellular wall that contain clearly recognized lining cells with very clear nuclei and there is no indication of cellular damage (Figure 18). Their lumen is empty with no sperm cells

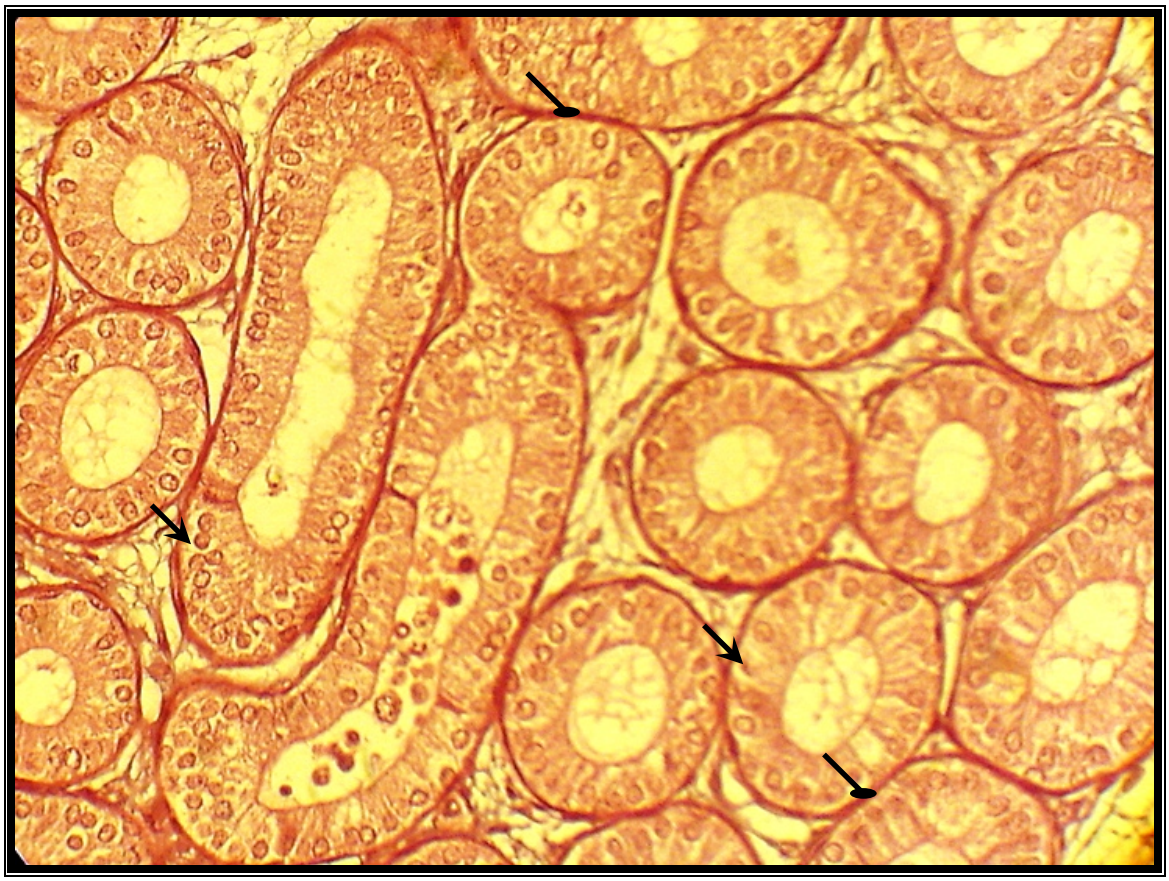


Figure 19: A histological picture of the epididymis treated with (5 mg/kg of b.w.) aspirin shows the tubules of the experimental group 1. (Haematoxylin and Eosin X400).

- ↘ : This arrow indicates lining cells.
- ↘: Attachment points.

Sections of these tubules appear to be compact and attached in different spaces with small spaces between them, the overall diameter of the tubules are hence smaller in highly significant way than the control, and the tubules are showed cellular wall that contain clearly recognized lining cells with very clear nuclei and there is no indication of cellular damage (Figure. 19). Their lumen is empty with no sperm cells.

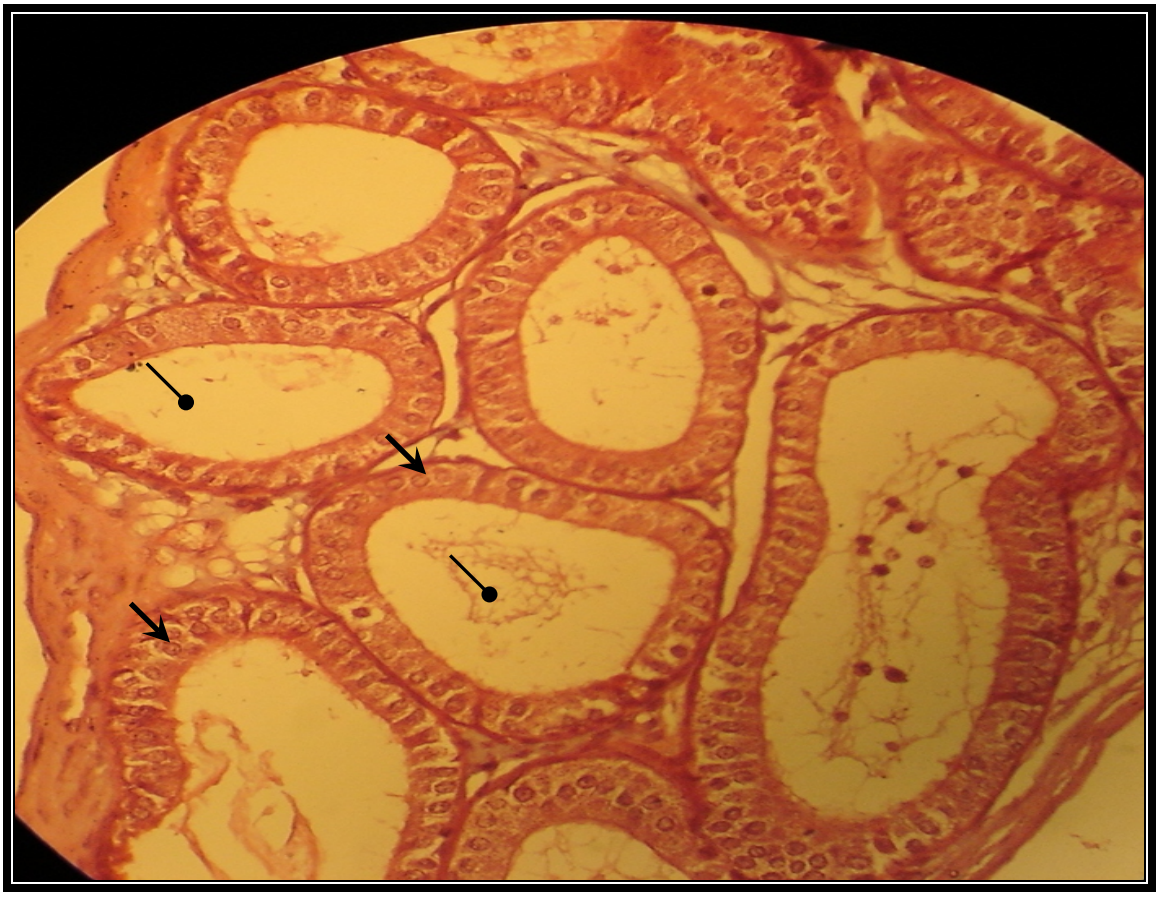


Figure 20: A histological picture of the epididymis treated with (25 mg/kg of b.w.) aspirin shows the tubules of the experimental group 2. (Haematoxylin and Eosin X400).

- ↘ : This arrow indicates height of the cells lining tubules.
- ↘ : This arrow indicates a wide and empty lumen.

Main structural changes in the epididymis in these animals include highly significant decrease in the height of the cells lining tubules, with wider empty spaces of both experimental groups when compared with the control and with each other (i.e. experimental group 2 decreases more significantly than experimental group 1), their lumen again appear to be empty, several empty spaces appear between epididymal cells, with no sperms (Figure. 20), the overall diameter of the tubules are hence smaller in highly significant way than the control.

3.4. Hormonal changes associated with aspirin (LH FSH and Testosterone)

Aspirin treated mice for ten days and killed on the 11 day showed the following (Figure 22):-

a- in experimental group 1 a significant decrease ($P < 0.05$), decrease was seen in FSH level only in experimental group 2 as compared to the control (Figure. 21).

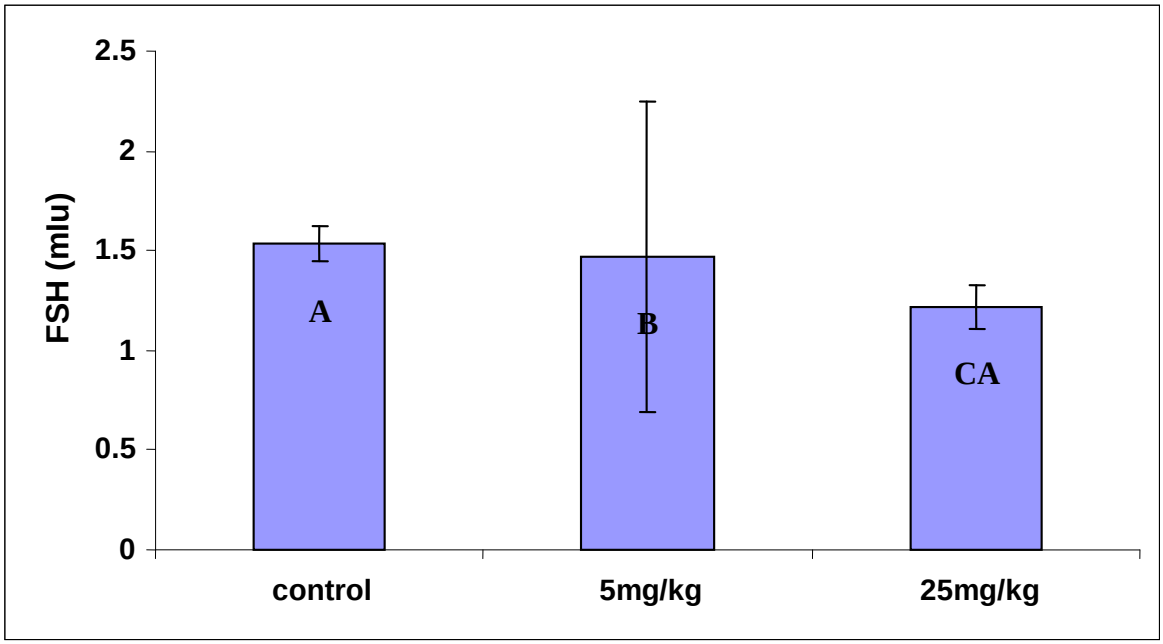


Figure 21: FSH hormone changes associated with aspirin administration to mice at concentration (5 mg/kg body weight twice/day) and (25 mg/kg body weight twice/day).

* Two letters = significant decrease ($p<0.05$).

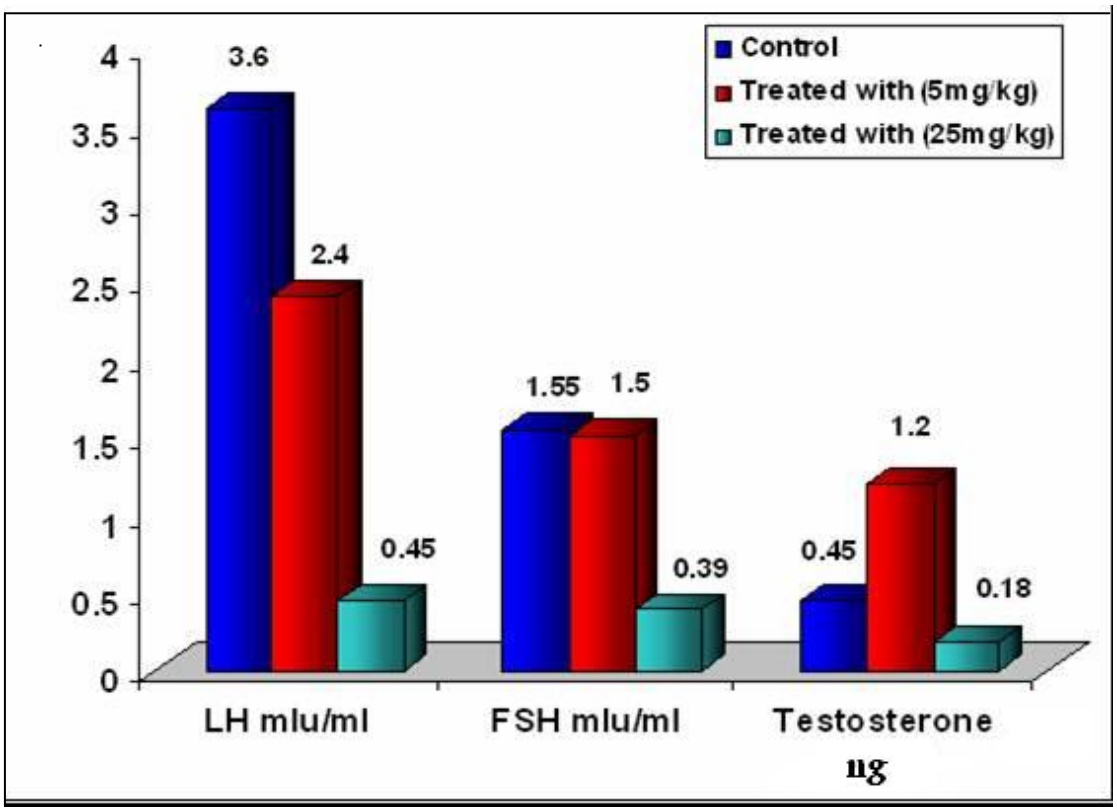


Figure 22: Hormonal changes associated with aspirin administration to mice at concentration (5 mg/kg body weight twice/day) and (25 mg/kg body weight twice/day).

This general figure for all hormones represents the decrease in all hormones as compared with control and with each other.

b- while LH hormone showed a highly ($P < 0.001$) decrease in both experimental groups as compared to the control, when experimental groups are compared to each other a highly significant decrease ($P < 0.001$) was seen in experimental group 2 as compared to experimental group 1 (Figure. 23).

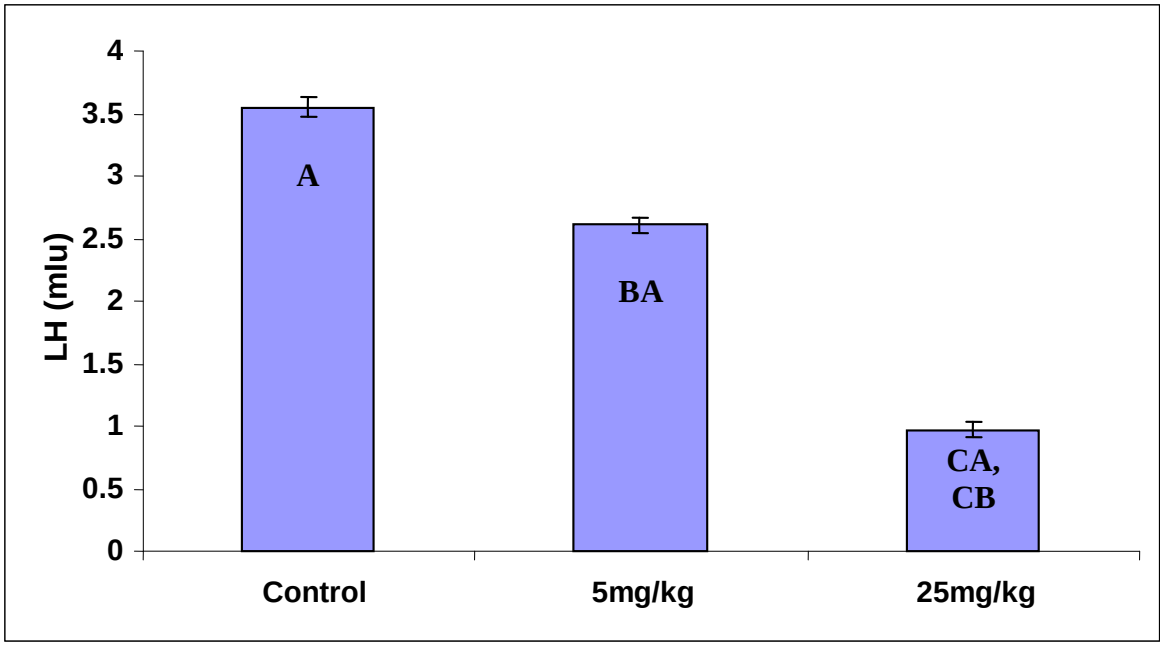


Figure 23: LH hormone changes associated with aspirin administration to mice at concentration (5 mg/kg body weight twice/day) and (25 mg/kg body weight twice/day).

* Two letters = highly significant decrease ($p<0.001$).

c- Testosterone level showed a highly significant decrease ($P < 0.001$) in experimental group 2 to both control and experimental group 1 (Figure. 24). However no significant changes were recorded in experimental group 1 as compared to the control. Several authors have recently shown that, administration of aspirin caused a significant decrease in both FSH and LH (Al-Rubaee, 2005). This decrease was suggested to be due to aspirin suppression of PGE_2 in the hypothalamus. This effect of aspirin seems to be induced through suppression of GnRH release when aspirin is given in a high dose (similar to us) (Yen *et al.*, 1999).

Also, Seely (1996) have shown that PGs play an important role in spermatogenesis from spermatogonia until spermatid formation. Suppression of PGs a limitation of their availability due to aspirin treatment in high dose

leads to blockage of sperm formation. Similar results have been obtained in the female mice (Al-Bayaty, 2002) who has shown that the administration of aspirin in a very high dose caused a complete cessation of ovulation associated with significant decrease in both (FSH and LH) this decrease was interpreted on the ground that it has been due to suppression of PGE_2 by aspirin centrally in the hypothalamus and locally on the ovarian level PGs, synthesis and release of testosterone is known to controlled by LH hormone, decrease in LH level theoretically should have induced and decrease in testosterone level due to removal of the negative feed back effect on LH hormone (Anonomous, 1999).

However testicular and epididymal weight changes must have rendered the testes unable to produce sufficient amount of testosterone and lack of response to decrease LH. Decreased testosterone production may be incriminated for the weight structural changes seen in the epididymis. Since testosterone is known to control epididymal growth and function (Al-Hasson, 1983). The decrease in testosterone production as a result of aspirin administration may be have deprived the body from the anabolic effect of testosterone and hence the decrease in body weight seen in the treated animals.

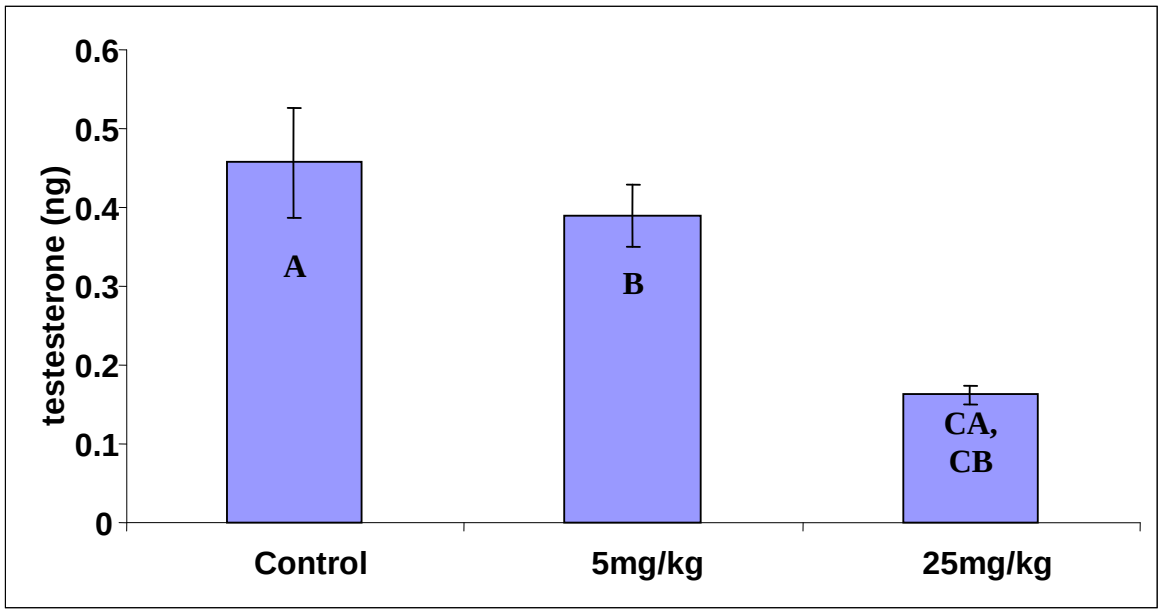


Figure 24: Testosterone changes associated with aspirin administration to mice at concentration (5 mg/kg body weight twice/day) and (25 mg/kg body weight twice/day).

* Two letters = highly significant decrease ($p<0.001$).

Summary

Aspirin is one of the most famous, cheapest, available and widely used drugs in the world in patient with a wide range of therapeutic uses for the treatment of inflammatory joint disease, prevention of thrombosis and many other causes due to its anti-inflammatory, analgesic antipyretic and antiplatelets effects. The present study was conducted as an attempt to study its effect on male reproduction to meagerness of scientific knowledge in this area. Aspirin was injected intraperitoneally to immature male mice at two doses: 5 and 25 mg/kg B.W. twice daily for 10 days (experimental group 1 and 2). Number of animals in each group was 15. Mice treated with normal saline were considered as control group. Results indicated that: testicular and epididymal weight, a significant decrease ($P < 0.05$) in testicular weight and in epididymal weight ($P < 0.001$) in both experimental groups as compared to the control. Structural changes in testes and epididymis have shown: Aspirin administration also caused a significant decrease ($P < 0.05$) in seminiferous tubules diameter associated with increase in empty spaces between them and thickness of seminiferous tubules. A similar decrease in epididymal tubular diameter and height of their cells. Changes in serum prepared from blood obtained by cardiac puncture: a significant decrease ($P < 0.05$) in serum FSH level in experimental group 2 as compared with control. A similar change was seen in other two remaining hormones (LH and testosterone) except that, the decrease in both experimental when compared with each other as with control was highly significant ($P < 0.001$). These results clearly indicate that aspirin when given at a low dose (5 mg/kg) or in much higher dose (25 mg/kg) for relatively long period (10 days) is risky when administered twice daily at least on the parameters studied on male mice reproductive system.

Supervisor Certification

We certify that this thesis was prepared under my supervision at the Department of Biotechnology, College of Science, Al-Nahrain University as partial requirements for the Degree of Science in Biotechnology.

Signature:
Supervisor:
Prof. Dr. Adnan Salih Al-Janabi

In review of available recommendations, I forward this thesis for debate by Examining Committee.

Signature:
Head of Biotechnology Department
College of Science
Al-Nahrain University

Dedication

To my

Father, who was trying the best to make me the best.

To my

Mother, who has the faithful heart with deep love.

To my

Dear sisters, brothers, Yasser, Muthana, Ahmed and Dhyaa for their patience during the period of the study and all my life.

To my

Lecturers for lightening my way with knowledge.

الى أغلى و أعز وأحلى ماأملك ..

آ لى ك ح ي ا ب

الى من وضعته وسأضعه تاج على رأسي ..

ك خ ى ك ح ي ا

الى من علموني قوة العزم والارادة ..

آ خ ه ت ى ئ إ غ ئ ؟

الى من شاركوني فرحي وحزني ..

آ خ ه ت ى ك ع ي ئ ة

بلال

١. الواجهة الانكليزية

٢. الايـــــــــة

٣. supervisor

٤. committee

٥. الاهداء

٦. Acknowledgement

٧. List of contents

٨. list of tables

٩. List of figures

١٠. Abbreviations

١١. summary

١٢. فاصـــــــل الاول

١٣. الفصل الاول

١٤. فاصـــــــل الثاني

١٥. الفصل الثاني

١٦. فاصـــــــل الثالث

١٧. الفصل الثالث

١٨. فاصـــــــل conclusion & recommendation

١٩. conclusion & recommendation

٢٠. فاصـــــــل references

٢١. references

٢٢. الخلاصـــــــــــــة

٢٣. الواجهة عربيـــــــــــي

الخـلاصـــــــة

يعتبر الاسبرين واحدا من اشهر وارخص الادوية واكثرها وفرة واستخداما من قبل المرضى في معالجة العديد من الامراض، كامرض المفاصل الالتهابي ومنع تكوين الخثر الدموية واستعمالات اخرى عديدة لكونه يعمل كمضاد للالتهابات ومسكن للالام وخافض للحرارة. وتعد الدراسة الحالية محاولة لدراسة تاثير هذا العقار على التكاثر الذكري، لاغناء المعرفة العلمية في هذا الجانب. وقد تضمنت الدراسة تقصي تأثير حقن عقار الاسبرين في الفئران الغير بالغة (كنموذج تجريبي) على اوزان الخصى والبرابخ بالاضافة الى دراسة تأثيره على التركيب النسيجي لهذين العضوين التناسليين المهمين. تم اعطاء الاسبرين عن طريق الحقن في التجويف البطني وبجرعتين (٥ ملغم \ كغم. وزن الجسم- جرعة قليلة) و(٢٥ ملغم \ كغم. وزن الجسم- جرعة عالية) مرتين في اليوم ولمدة عشرة ايام وسميت هاتان المجموعتان (المجموعة التجريبية ١ والمجموعة التجريبية ٢). بلغ العدد الكلي للفئران المستخدمة ٤٥ فأراً (١٥ لكل مجموعة) وحقنت مجموعة السيطرة بالمحلول الملحي الفسلجي.

بينت نتائج هذه الدراسة حصول انخفاض معنوي ($P < 0.05$) في اوزان الخصى في المجموعتين التجريبيتين بالمقارنة مع السيطرة وكان الانخفاض عالي المعنوية ($P < 0.001$) في اوزان البرابخ. عند فحص التغيرات النسيجية لخصى وبرابخ هذه الحيوانات اتضح وجود انخفاض معنوي ($P < 0.05$) في اقطار النبيبات المنوية في الحيوانات التجريبية بالمقارنة مع السيطرة. وكان هذا الانخفاض عالي المعنوية ($P < 0.001$) في اقطار البرابخ في كلا المجموعتين عند المقارنة بمجموعة السيطرة. كما سجلت زيادة معنوية ($P < 0.05$) في معدل الفراغات مابين النبيبات المنوية صاحبه انخفاضاً معنوياً في سمك الجدار الخلوي المبطن للنبيبات المنوية في كلا المجموعتين التجريبيتين بالمقارنة مع السيطرة، وكانت التغيرات النسيجية في ارتفاع الخلايا المبطنة للبربخ مماثلة للتغيرات النسيجية في جدار النبيبات المنوية حيث انخفضت وبشكل عالي المعنوية ($P < 0.001$) عند المقارنة مع السيطرة او المجموعة الثانية بالمقارنة مع الاولى. لقد كانت هذه التغيرات انعكاسا للتغيرات الحاصلة في مستويات الهرمونات التكاثرية الرئيسية في مصل الدم حيث انخفض بشكل معنوي ($P < 0.05$) الهرمون المحفز للجريبات (FSH) في المجموعة التجريبية الثانية مقارنةً مع السيطرة. اما الانخفاض في مستوى الهرمون اللوتيني (LH) في المجموعتين التجريبيتين بالمقارنة مع مجموعة السيطرة فقد كان الانخفاض عالي المعنوية ($P < 0.001$)، ليس هذا فقط وانما اتسع الانخفاض ليشمل المجموعة الثانية بالمقارنة مع الاولى. ان هذا الانخفاض المعنوي في هرمون LH

نتيجةً لحقن مادة الاسبرين ترافق معه انخفاضا عالي المعنوية (P< 0.001) في المجموعة التجريبية الثانية بالمقارنة مع المجموعة التجريبية الاولى وكذلك السيطرة ولم يلاحظ وجود فرق معنوي مابين مجموعتي السيطرة والتجريبية الاولى على الرغم من الانخفاض غير المعنوي في المجموعة التجريبية الاولى. ان هذه النتائج جميعاً مترابطة مع بعضها البعض ويكمل بعضها البعض الاخر وهي تشير جميعا الى ان الاسبرين عند اعطائه بالجرعة القليلة (٥ ملغم \كغم) او في جرعة كبيرة (٢٥ ملغم\كغم) ولفترة طويلة (١٠ ايام) يكون ذا تأثير سلبي على الجهاز التناسلي الذكري في حالة اعطائه يومياً على الاقل بالنسبة للجوانب التي تمت دراستها على الجهاز التناسلي الذكري للفئران. ان المزيد من الدراسات مطلوبة لتوضيح الكثير من جوانب هذه التاثيرات وغيرها نظراً لاهمية هذا العقار وشيوع استخدامه في البشر.