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Contribution to the study of the effects of an aqueous extract of *Myristica fragrans* Houtt "Nutmeg" on male *Albino Wistar* rats fertility

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In the name of Allah, The Most Beneficent, The Most Merciful.

All the gratitude goes to Him, for I would get nowhere without His guidance and blessing.

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To all the researchers around the world

To my future self, I'm sure that you will be proud of me

Ahlem

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AMP Kinase

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Michigan Cancer Foundation-7

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INTRODUCTION

Myristica fragrans Houtt it's an evergreen tropical plant belongs to the family of *Myristicaceae*. Its fruit is a bare corn with a succulent pericarp and winking mace around the seed. the seed is hard, fleshy and abounds with oil. the kernel is nutmeg that's used commercially and has medicinal properties.

Nutmeg is widely used as spice and in alternative medicine it has been reported to have aphrodisiac, stomachic, carminative, tonic, nervous stimulant, aromatic, narcotic, astringent, hypolipidemic, antithrombotic, antifungal, antidysentery and anti-inflammatory properties. It is reported to be useful in paralysis too and increases blood circulation.

Although studies have reported that nutmeg has a therapeutic effect, this cannot be undeniable Shows nutmeg toxicity, which may result from exposure to high doses and/or exposure combinations extracted by various methods.

Very few studies have been conducted to examine the effect of nutmeg on fertility or reproduction in general, which makes it interesting to study its effects on fertility

According to previous references and studies, the effects of nutmeg as an aphrodisiac were studied and gave positive results **(Tajuddin, *et al.*, 2005)**, and according **to Hussein & Ayoub., (2007)**it's oil extract was studied on testicular functions in rats exposed to 1% hydrogen peroxide for 21 days, and the results showed that Oral administration of nutmeg oil extract at 500 mg/kg dose produced a highly significant decrease in weight of testis and sperm count and percentage of viable and normal sperm as compared to hydrogen peroxide treated group. Also, the mean number of spermatogonia reduced to a significant level as compared to hydrogen peroxide treated group and control. Another study proved the antifertility and recovery effect of *Myristica fragrans Houtt* oil in male albino rats **(Meera, *et al.*, 2009)**.

Therefore, our current study speculates reasonably, and according to previous studies, that the use of nutmeg may lead to a decline in male fertility in relation to dose, duration of exposure, and/or extraction type.

The aim of our work is to study the chronic effects of *Myristica fragrans* "Nutmeg" aqueous extract on some male fertility parameters in male albino rats, also its effect on other selected organs and biochemical analysis.

BIBIOGRAPHIC SUNTHESIS

CHAPTER I

MYRISTICA FRAGRANS HOUTT "NUTMEG" AND MALE FERTILITY

1-Botanical description

Myristica fragrans Houtt, also known as nutmeg tree, is an evergreen tropical plant that belongs to Myristicaceae family. The nutmeg tree is a small, slow growing tree that can reach up to 20 meters in height with a spreading and greyish-brown or black branches, its leaves are glossy above, dark green, aromatic, alternate, glabrous and petioled **(Periasamy, *et al.*, 2016)**.The tree has small, pale, yellow flowers, which are usually dioecious with a small axillaries, arranged in clusters and sometimes forked. This dioecious plant propagates both sexually and asexually, the latter being the standard. **(Haldankar, *et al.*, 2007).**

The tree bears fruit all year, but the best time to harvest is between April and November. Nutmegs fruit is a large, fleshy, yellowish-brown drupe that splits open when ripe, revealing the seed which is commonly known as nutmeg. The nutmeg seed has an inner oval-shaped, hard, fleshy kernel abounds in oil and an outer red aril called mace. the kernel is nutmeg, both nutmeg and mace used as spice also have medicinal properties **(Wallis, 1985).**

Myristica fragrans is native to the spice Islands of Indonesia and is widely cultivated throughout the tropics, including India, Sri Lanka, USA, and South Africa. **(Francis, *et al.*, 2019).**

Figure 1 : Morphological identification of *Myristica fragrans Houtt*. (a) The young tree; (b) leaves of the plant; (c) fruit; (d) seed; (e) rind; (f) kernel (nutmeg); (g) mace (aril); (h) ground powder of mace; and (i) mace essential oil. **(Ashokkumar, *et al.*, 2022).**

The classification of this plant is as follows **(Kumari, *et al*., 2021):**

Kingdom : Plantae
Infrakingdom : Streptophyta
Devision : Tracheophyta
Class : Magnoliopsida
Order : Magnoliales
Family : Myristicaceae
Genus : Myristica
Species : *Fragrans*

Photo 1 : *Myristica Fragrans* seed.

2-Utilizations of *Myristica fragrans*

2-1-In alternative medicine

However, in Indian Ayurvedic medicine, nutmeg has been used to treat diarrhea, stomach cramps, parasites, paralysis, rheumatism, anxiety, nausea and as an aphrodisiac **(Gils & Cox, 1994) (Ziyatdinova, *et al*., 2016)**. Additionally, the nutmeg plant has been used in traditional Pakistani medicine to treat hypertension **(Malik, *et al*., 2018)** . Many believe that nutmeg increases blood flow and helps stimulate the cardiovascular system, thus easing heart problems. It relieves vomiting, diarrhea, gas and stimulates appetite. It is also used for respiratory disease. It is often found as an ingredient in cough syrups. It is said to help with asthma. Although nutmeg has many health benefits, care should be taken not to exceed the minimum dose as it is toxic and can cause serious health problems **(Burdock & Carabin, 2007).**

2-2-In cooking and aromatic industries

Nutmeg is a fragrant and thick spice that has long been known for its aroma and is one of the most important spices in Indian cuisine **(De Melto & Fray, 2005)**. This spice is used as a savory flavoring and to give food its saffron color all grains are often preferred over powders because they contain more essential oils and hulls, imparting a richer taste, flavor and freshness to the recipes. Often the dried beans are ground or thoroughly ground before been added at the last minute from cooking. However, ingesting can cause difficulty concentrating, sweating, heart palpitation and body aches. In severe cases hallucinations and delirium can occur **(Orabi, *et al*., 2000)**. Therefore, the consumed doses must be observed.

Nutmeg essential oil has many uses thanks to its olfactory and cosmetic properties. Nutmeg essential oil is a common ingredient in perfumes, massage products, soaps, skin and hair care products. Its spicy scent, in particular, is popular in men's products such as shave him creams and beard oils **(Parthasarathy, *et al.*, 2008).**

3-Chemical composition

Phytochemical screening of nutmeg extracts revealed the presence of alkaloids, flavonoids, and anthraquinones as shown **Table 1**. **(Olaleye, *et al.*, 2006)**

Table 1: Phytochemical constituents of aqueous extract of *Myristica fragrans* **(Olaleye, *et al.*, 2006)**

Constituents	**Result**
Alkaloid	+
Saponins	+
Tannins	-
Anthraquinones	+
Cardiac glycoside	+
Flavonoids	+
Phlobatanins	+

(+) present, **(-)** absent

The main chemical constituents of nutmeg are alkylBenzene derivatives (myristicin, elemene, safrole) myristic acid, A-pinene, terpenes, β-pinene, and trimyristin. **(Qui, *et al.*, 2004). (Yang, *et al.*, 2008)**.

Nutmeg contains about 10% essential oils, mainly composed of terpenes hydrocarbons (sabinene and pinene), myrcene, phellandrene, camphene, limonene, terpinene, myrcene, pcymene and other terpene derivatives **(Jaiswal, *et al.*, 2009).**

The presence of each chemical compound varies according to the soil type, location and season. **(Ashokkumar, *et al.*, 2021) (Ashokkumar, *et al.*, 2022).**

Nutmeg also produces nutmeg butter, containing 25-40% fixed oil, it is semi-solid reddish-brown fat Aroma with nutmeg. Nutmeg contains trimyristin, oleic acid, linoleic acid and resinous substances. Nutmeg Fatty Oil Butter for perfume and topical for sprains and rheumatism**. (Peter, 2001).**

4-Biological effects

Nutmeg is a resource of many biologically active compounds with different pharmacological properties, which encourages researchers to discover diverse and innovative therapeutic applications that benefit humanity. Many nutmeg seed extracts have shown different activities such as hepatoprotective activity, antioxidant, memory enhancing, Anticancer, aphrodisiac, antidiabetic, hypolipidemic and cholesterol-lowering effects, Antimicrobial, and anti-inflammatory. **(Tripathi, *et al.*, 2016)**

We have taken examples of some of its biological effects according to the ongoing studies as follows

4-1-Cytotoxicity

Phytochemical screening of different nutmeg extracts revealed the presence of alkaloids which comprising a large group of nitrogenous compounds are widely used as cancer chemotherapeutic agents. **(Tolulope, *et al*., 2013)**

According to **(Mary, *et al*., 2012)** it was observed that the addition of different concentrations of nutmeg essential oils against Michigan Cancer Foundation-7 (MCF-7) breast cancer cell line and A-357 epidermal Skin cancer cell line are cytotoxic. In other study by 36 they found that nutmeg's methanolic extract leads to Jurkat leukemia T cell line death through an associated mechanism SIRTI mRNA down regulation **(Chirathaworn, *et al*., 2007).**

4-2-Anti-obesity activity

In the ongoing search for novel plant-derived AMP Kinase (AMPK) activators, whole extract of nutmeg was found to activate the AMPK enzyme and prevent overfed mice from gaining adipose tissue mass, body weight, LDL and glucose levels increased in groups of mice as compared to control group. **(Nguyen, *et al.*, 2010).**

4-3-Sedative and sleep Enhancing property

Preclinical evaluation of nutmeg seeds aqueous extract was done for sedative and hypnotic activity in rats and mice. Based on **(Chaitra, *et al.*, 2020)** study's results It can be concluded that MFAE (*Myristica Fragrans* seed aqueous extract) in a dose 200mg/kg shows significant sedative and sleep enhancing activity.

4-4-Antimicrobial effects

The extract of *Myristica fragrans* showed antimicrobial activity against *Escherichia coli, Saccharomyces cerevisiae, Bacillus subtilis*, also the extract was not effective against *Salmonella typhi* and *Helicobacter pylori* because of drug resistant also an inhibition effect against survivorship of *Yersinia enterocolitica* and *Listeria monocytogenes* in broth culture and Iranian barbecued chicken **(Jaisual, *et al.*, 2009).**

4-5-Antioxidant activity

As a comparation of popular food antioxidants like butylated hydroxytoluene (BHT) (E-321), propyl gallate (E-310), butylated hydroxyanisole (BHA) (E-320) against antioxidants properties of some spices. According to deoxyribose assay nutmeg, anise and licorice reveal the strongest protection. Nutmeg, propyl gallate, ginger and licorice ameliorated the stability of oils and fats against oxidation. The antioxidants capacity of BHT was found less than nutmeg depending on the Trolox equivalent antioxidant capacity (TEAC) assay **(Murcia, *et al.*, 2004).**

4-6-Anticonvulsant Activity

Nutmeg essential oil was also found to have a pronounced anticonvulsant activity against different size of animals and also epilepticus status, the study reveal that the anticonvulsant effect because of decreasing the dopaminergic transmission **(Sonovane, *et al.*, 2002).**

Myristica fragrans exctract showed antispasmodic effect on tonic extension of the hind limbs induced by electric shocks. It showed a dose-dependent anticonvulsant effect on pentylentrazole-induced tonic seizures. This delayed the onset of strychnine-induced hind limb tonic extensor spasm. In addition, pentylenetetrazole and bicuculline induce clonic seizures, so low doses were anticonvulsant, whereas high doses were mildly proconvulsant **(Wahab, *et al.*, 2009)**.

4-7-Analgesic effect

It has reported that alkaloids extracted from *Myristica fragrans* seeds in dose of 1 g/Kg.bw caused a modest and reduced the number of writhing models in female but not in male mice. **(Hayfaa, *et al*., 2013)**

5-Toxicity

In 1576 the first nutmeg intoxication was described, a pregnant woman consumed approximately 70-84g of nutmeg to induce inebriety **(Stein, *et al*., 2001)**. Due to nutmeg hallucinogenic effects numerous cases of intentional poisoning was recorded, nutmegs poisoning cases was divided into two groups, intentional abuse and non-abuse **(Rahman, *et al*., 2015) .**

Myristicin is the major component that is responsible for nutmeg's intoxication, when consumed in a large portion and its frequent usage can cause fatal incidents resulting in organ damages, impact on the cardiovascular system, causes Fatty degeneration in the liver, also it's known for its hallucinogenic effect and neurotoxicity too **(Sangalli & Chiang, 2000) (Gupta & Rajpirohit, 2011).**

Symptoms of nutmeg poisoning usually begin 3 or 6 hours after consumption or exposure to it. Those who are poisoned with nutmeg show several symptoms, including facial flushing, tachycardia, hypertension, dry mouth, blurred vision, psychoactive hallucinations, feelings of euphoria (unreality) and delirium which usually disappears 24 to 36 hours after exposure **(Gupta & Rajpirohit, 2011).**

It has reported that 5g of nutmeg powder considered as toxic dose while about 2 nut can induce psychogenic effects **(Stein, *et al*., 2001).**

6-Myristica fragrans and male fertility

6-1-Aphrodisiac activity

Nutmeg has long been mentioned in Unani and alternative medicine for its aphrodisiac properties, it has reported that 50% nutmeg ethanolic extract have an aphrodisiac effect on male mice and albino rats in a dose 500 mg/kg.bw, there was a significant increase in sexual behaviour and activity, it has observed that there was an increase in mounting, intromission frequency, in libido

and potency too. And also lends support to the claims for its traditional usage as sexual function enhancing medicine **(Tajuddin, *et al.*, 2003) (Tajuddin, *et al.*, 2005).**

6-2-Antifertility effects

In contrast to some studies that demonstrated the effectiveness of nutmeg in treating some male sexual disorders, some studies demonstrated the anti-fertility effects of nutmeg on male rats.

It has reported that Oral administration of nutmeg oil in different concentrations have antifertility effects on male albino rats, there was a significant decrease in some fertility parameters which could recover after terminating the treatment **(Meera, *et al.*, 2009)** and according to **Hussain ., (2007)** study Its oil extract was studied on testicular functions in rats exposed to 1% hydrogen peroxide for 21 days, and the results showed that Oral administration of nutmeg oil extract at 500 mg/kg dose produced a highly significant decrease in weight of testis and sperm count and percentage of viable and normal sperm as compared to hydrogen peroxide treated group. Also, the mean number of spermatogonia reduced to a significant level as compared to hydrogen peroxide treated group and control.

CHAPTER II:

LABORATORY RAT'S REPRODUCTION AND FERTILITY

Introduction

"laboratory," rat (*Rattus norvegicus*) has been used in behavioral, neural, physiological, and other researches for more than a century. The evolutionary history of this species is often dismissed as unimportant in psychological and biomedical research because the aim is not to understand evolutionary biology but rather to use the rat as a model system to investigate a specific aspect of organismal biology. Laboratory rats have an important role in reproductive researches including fertility studies, in this chapter we aim to make a small review on laboratory rat's reproduction and fertility especially male rats.

Taxonomic classification of the Laboratory Rat **(Mark, *et al*., 2006)**.

Kingdom: Animalia

Phylum: Chordata

Class: Mammalia

Order: Rodentia

Family: Muridae

Genus: Rattus

Species: *norvegicus*

Strain: *Albino Wistar*

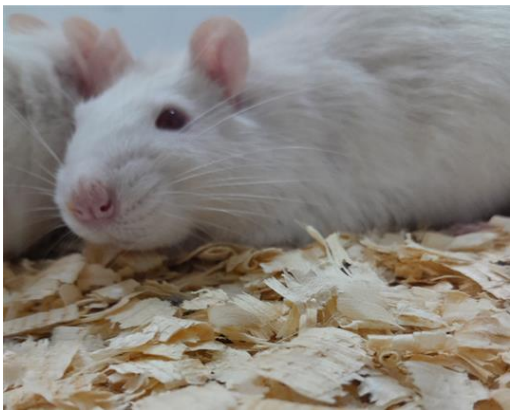

Photo 2 : *Albino Wistar* rat.

1-Sex determination

Is the most easily in adult rats by assessing the anatomical structure of the perineal region, it should be noted that male is still capable to regress the testicles into the abdomen. In addition, we can determine the sex by the distance between the anus and the urethral opening. The male anogenital distance is bigger than that of the female. In pups less than two week , sex determination may be more difficult due to the relatively small size of the rat pups, we can't use testicles in pups before weaning to aid in sex determination because testicle usually descend into the scrotum after about 15 days **(Russell, 1992)** , observation of the udder can be used to identify females, since males have no nipples **(Cowie, 1984)** .

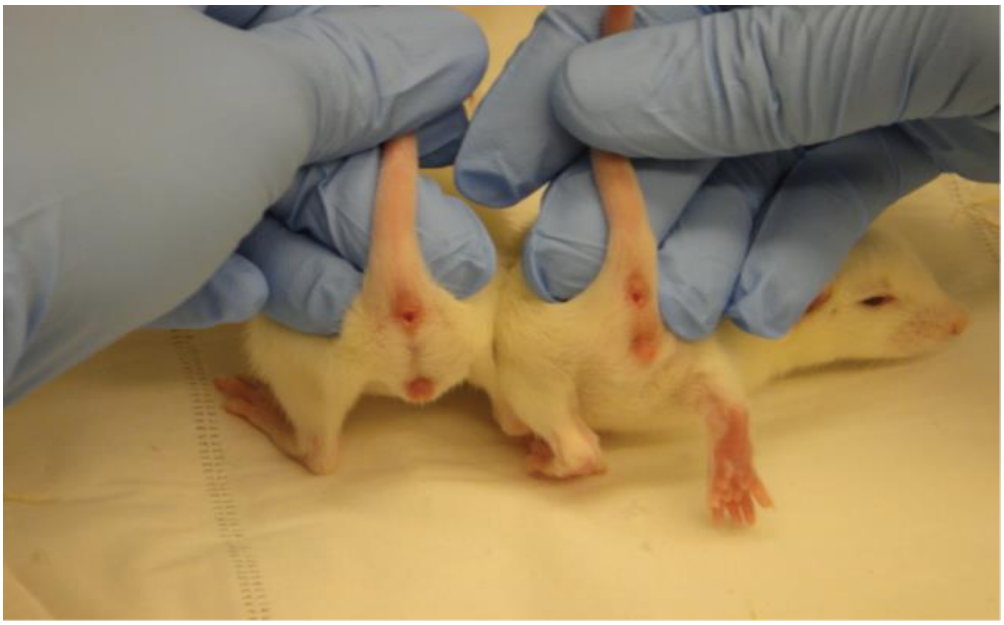

Figure 2 *:* anogenital distance in the preweaning Sprague-Dawley male rat (left) compared to the female (right). **(Patrick & Jason, 2013)**

2-Puberty and male reproductive development

Sexual maturity is the ability to produce viable young, it occurs as a result of the descent of the testicules into scrotum, and the beginning of spermatogenesis, its approximately the 45th day after birth.

The age of puberty varies according to breeds and also influenced by a number of other factors

According to **Bennett and Vickery., (1970)** it was observed that rat becomes sexual mature when it reaches 50% of its mature body weight.

The spatial developmental change in spermatozoa seen over the seminiferous tubule is called the spermatic wave. The spermatogenic cycle lasts approximately 12 days, and must be completed four times from the first division of a spermatogonia to produce spermatozoa in the tubule lumen **(Setchell, 1982)**. The time from spermatogonia to spermatozoa is relatively consistent and requires 48 to 52 days in the rat **(de Krester, 1982)**. Spermatogenic cycles begin in very young rats, but the cycles are incomplete and irregular until puberty, when spermatogenesis becomes more coordinated and regular. From the lumen of the seminiferous tubules, spermatozoa collect in the tail of the epididymis and take approximately eight days to traverse the head of the epididymis **(Russell, 1992)**.

3-Reproduction and fertility

3-1-Mating behaviour

The most important thing in mating behavior in rats is receptivity, the receptivity between the estrus female and male.

Lordosis is the characteristic mating behavior of female rats, both estrogen and progesterone are involved in this reflex but also estrogen alone is sufficient to induce the behavior **(Maeda , *et al*., 2000).**

Testosterone is essential to the exhibition of mating behavior in male rats, so ani changes in testosterone levels can affect rat's sexual behavior, it was mentioned that castrated male rats show no sexual behavior **(Meisel & Sachs , 1994).**

Olfactory cues from pheromones are also critical to male sexual behavior **(Nelson, 1995).** Auditory stimuli play important roles in the reproductive behavior of both sexes. A 50-kHz vocalization is produced by both males and females during copulation. This call is produced by males in response to females in estrus, and by the female to solicit male attention **(Maeda , *et al*., 2000)**. The male rat also produces a 22-kHz vocalization in the post-ejaculatory refractory period **(Barfield & Thomas, 1986)**. Copulation in rats occurs most often during the latter portion of the dark cycle **(Mercier, *et al*., 1987).** The male initiates mating behavior with genital sniffing of a female in estrus. The receptive female displays hopping and ear quivering, resulting in male mounting, which in turns solicits lordosis from the female. Mounting behavior consists of combinations of intromission and ejaculation, including mounting without intromission; mounting with intromission but without ejaculation, frequently with a backward lunge; and mounting with intromission and ejaculation. An intromission typically consists of

two to nine pelvic thrusts and lasts 0.3 to 0.6 seconds **(Bennett & Vickery , 1970)**. Ejaculation is typically preceded by 3 to 44 intromissions, and a non-responsive refractory period occurs after ejaculation. Mating continues intermittently for up to three hours, until 3 to 10 ejaculations have been achieved (**Mark, *et al.*, 2006)**.

Mating can be confirmed by the presence of sperm in a vaginal smear, by observation of a vaginal plug, or by direct observation of the mating behavior. The vaginal plug does not persist as long in the rat as it does in the mouse, and thus the lack of a plug is not a reliable indicator that mating did not occur. Vaginal smears that detect sperm-positive animals are commonly used to confirm mating, and a 90 to 94% correlation between sperm-positive vaginal smears and pregnancy has been reported **(Baker, 1979).**

3-2-Mating forma

- **Monogamous Stable-pair Mating**

It involves placing a male and female in a breeding cage, where they spend their reproductive lives together. for descendants records this type is the easiest one **(Mark, *et al.*, 2006)**.

- **Polygamous Stable Trio Mating**

This system is achieved by placing one male with two females in the same breeding cage **(Mark, *et al., 2006)* .**

- **Polygamous Harem Mating**

In this system of mating format more than three females placed with one male in the same breeding cage **(Mark, *et al.*, 2006)**

Both advantages and disadvantages of different mating forma are shown in table n° 2.

Table 2 : Advantages and disadvantages of different mating forma **(Mark, *et al.*, 2006).**

Mating format	Advantages	Disadvantages
Monogamous stable-pair mating	• Mating occurs during postpartum estrous period • Lower labor intensity • Descendants will have identical parents • Easiest to keep descendants records	
Polygamous, stable trio mating	• Mating occurs during postpartum estrous period • Multiple lactating dams	• Require large cages • Decrease in the reproductive performance of the cage • More labor intensive • Difficult to keep descendants records
Polygamous harem mating	• A few number of males to mate with the largest number of females	• More labor intensive • Difficult to keep descendants records

4-Factors affects rats fertility

It is known that there are very wide variations in terms of reproductive performance among different stocks, lines and strains of experimental rats. Obviously, many of them are a reflection of Multifactorial differences in genotype that appeared in a more or less incidental during selection for other characteristics. An example of these variations is that of the superior fertility of male ACI rats on that of male Sprague-Dawley rats of the same age; this particularity is correlation with ACI rats living six months longer than Sprague Dawley **(Cameron, *et al.*, 1982).**

Other examples of genetically inherited differences in fertility may be related to single gene effect such as the significant reduction litter size of rats with jaundice (d/d) (Gun) compared to females free of jaundice (+/d) of the same strain **(Davis & Yeary, 1979)**.

It was reported that male rat's pups from large litters (14pups/mother) They have little weight and had delayed maturation of the testes and hypothalamus than males from younger litters (6 pups/mother). The augmentation in food intake prior to weaning did not show the same changes in the hypothalamus and testes for pups from large litters **(Bourguignon, *et al.*, 1992)**. The previous information suggests that caring pups from large litters can have a positive effect on the later fertility of males in the litter. **(Mark, *et al.*, 2006).**

Rats are a constant breeder in the laboratory, showing no signs of seasonality. female held in a constant light exposure in a 12-hourlight/12-hour-dark cycle as result the vaginal opening six days earlier **(Fiske, 1941)**Continuous light has also been shown to induce ovarian cysts and prolonged estrus **(Shirley, 1978)**; **(Maeda , *et al.*, 2000)**. Also Changes in photoperiod can alter reproductive characteristics in rats **(Clough, 1982).**

According to previous studies it was resulted that reproductive development and future fertility varies with any modification of nutritional status during pre- and post-natal development also. As examples, a fast of 48 hours duration at the start of estrus decreases LH secretions and prevents ovulation in rats **(Maeda , *et al.*, 2000)**; **(Santti, *et al.*, 1998)** investigated the effects of phytoestrogens on male rats. No effects were noted at doses similar to those in soybean feed. However, artificially high doses induced DES-like lesions. Exposure of male rat pups to phytoestrogens during lactation decreased testicular but not serum testosterone near weaning and caused altered sexual behavior in adulthood **(Santti, *et al.*, 1998)**. Thus, the extent of resulting reproductive changes varies with modification of nutritional plane and depends on the age at onset, chronicity, and severity of undernutrition

Chemical colitis resulted in retardation puberty in male, smaller gonads, and a lower plasma testosterone level compared with control, Colitis also retard puberty in females and disrupts the estrous cycle **(Azouz, *et al.*, 2001)**Also colitis increase malnutrition in rats. this proves the strong link between nutrition, health status and fertility.

Exposure of rat's testes to 43°C for 30 minutes leads to decline spermatogenesis and loss of testes weight by 50% **(Setchell, 1982)**. In one of the other studies, an increase in embryo mortality was observed, and a decrease in the size of rat's pups and their growth rate as a result of high temperatures, so this proves the sensitivity and influence of fertility by temperature especially and the surrounding environment in general **(Pucak, *et al.*, 1977).**

MATERIALS AND METHODS

1-First part: Preparation of the hot aqueous extract

1-1-Materials and products

Nutmeg was purchased from the Herbalist of downtown.

Distilled water, Erlenmeyer, beaker, funnel, watch glass, filter paper, mortar and pestle or a blender, magnetic stirrer, balance, thermometer, oven, Centrifuge

1-2-Grinding

After a good cleaning to remove impurities and dirt residue, the nutmeg is dried**,** after it has completely dried, the outer casing is removed to facilitate the grinding process wish carried out by using electrical blender until obtaining a homogeneous powder of nutmeg, then kept in tightly closed bottles until use.

1-3-Preparation of the hot aqueous extract

The aqueous extract prepared according to **(Tolulope*, et al*., 2013)** and **(Chaitra*, et al*., 2020)** with some changes.

Quantity of 900 g nutmeg's powder was used to achieve this experiment; the aqueous extract was prepared by soaking 10 grams of nutmeg powder in 100 ml of boiled distilled water at a temperature of 100°C.

The solution was then left under continuous mixing for 24 hours at 200 rpm/min, then filtered by Whatman filter paper.

After filtering, the extract centrifuged for 20 min and 3600 r/min.

During the entire experiment, the glassware was wrapped in aluminum foil to avoid exposing the extract to light and air.

The solvent was removed by oven at 50°C in petri dishes than the yield from the extract will be collected and calculated.

1-4-Determination of the yield of dry extracts

The yield is the quantity of dry extract obtained from the extraction of the vegetable powder. It is expressed in percentage or without unit. In practice, we reported the mass of the extract on the mass of the vegetable powder used for the extraction which we multiplied by 100.

This results in the following formula

$$\% = \frac{W1 \times 100}{\mathrm{W2}}$$

W1: weight of dry extract (g)

W2: weight of dry plant (g)

2-Second part: Protocol design

2-1-Animals

In this study we work on male *Albino Wistar* rat strain, which obtained from the animal warehouse of university of Constantine1.

The animal's selection based on our study's experimental protocol, which required healthy adult males with sexual experience.

The rats were divided into two groups, each group containing 6 rats, and they were numbered with a permanent pen. Each 3 rats were placed in one cage.

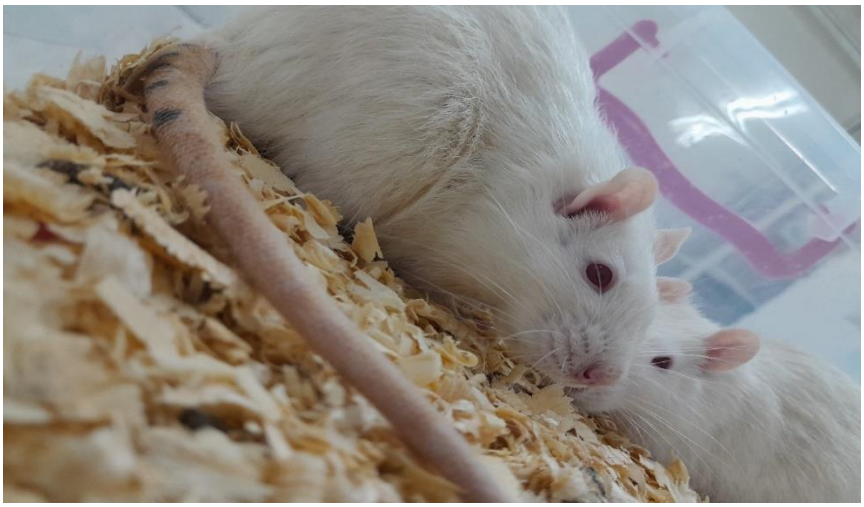

Photo 3 : Male *Albino Wistar* rats.

Group 1: served as a control group received 1.5 ml/kg body weight of distilled water orally, daily for 33 days at 10 am.

Groups 2: were received oral suspension of the nutmeg's extract in a dose 400 mg/kg body weight daily, for 33 days at 10 am.

2-2-Doses preparation

Dose of *Myristica fragrans* aqueous extract (MFAE) was given according to **(Tolulope, et al., 2013)**, it was chosen because it's the most effective dose with the least toxicity.

In an acute oral toxicity study for MFAE on male *Albino Wistar* rats, it was found that MFAE safe in a dose 1000 mg/kg body weight, MFAE showed no over signs of toxicity or lethality **(Mehamed , *et al*., 2013)**.

The MFAE was tested for solubility in distilled water, since it was soluble, distilled water was used as vehicle.

Photo 4 : Method of restraining and gavage.

2-3-Assessment of food consumption

Diet is an important factor that greatly influences the reproductive performances, its therefore important to control it for better results interpretation.

Thus, to assess food consumption, the quantities of food distributed and rejected were weighed daily.

Individual food consumption is determined by the following formula:

$$Fi = \frac{\mathrm{Fd} - \mathrm{Fr}}{\mathrm{N}}$$

Fi: Quantity of food intake

Fd: Quantity of food distributed

Fr: Quantity of food refused

N: Number of subjects

The comparison between the average food consumption of the animals having received the nutmeg aqueous extract and that of untreated animals (control group) will make it possible to know if the extract has any effect on food consumption.

2-4-Observation of any behavioural changes

In the main time of our study, we tried to observe any abnormal behavior changes on rats.

we take in consideration

- any signs of acute or chronic pain (vocalization, hustle, reducing activity ...)
- stress behavior (increased respiration, exophthalmia, piloerection …)
- Overt signs of acute toxicity (Salivation, convulsions, lachrymation …)

2-5-Dissection and testosterone dosage

After 35 days of treatment, all animals were starved overnight.

Before dissection, the rats are weighed and then sacrificed with chloroform anesthesia.

Blood is taken from the hepatic portal vein. And collected in lithium heparin tubes without missing and confirming tubes identification, so that control group tubes marked with “TR” and the treated group marked with “MFR”.

2-6-Study of body weight and weight of sexual or selected organs

The body weight of the animals is recorded every three days throughout the treatment period, with a balance. After dissection, a macroscopic examination of the organs in place is carried out, then they removed, cleaned and cleared fat, rinsed with physiological water then weighed with a balance (± 0.0001g) and finally stored in 10% formalin for further histological study. The organs concerned are the testicules, epididymis, the kidneys and the liver.

The evolution of the difference between initial and final body weight were calculated for all the treatment days.

2-7-Sperm quality study

2-7-1-Obtaining epididymal sperm

Sperm was obtained by making a small cut with scissors on the distal section in the head of the epididymis. The obtained sperm drop was diluted in 5 ml warm physiological water (37°C) the water warmed for better dissemination of spermatozoa **(Kuriyama et al, 2005).**

To assess respectively: mobility, velocity, viability and sperm concentration

2-7-2-sperm velocity

sperm velocity is calculated by the following formula:

$$V = \frac{\mathrm{D}}{\mathrm{T}}$$

V: Sperm velocity (µm/sec)

D: the distance between 2 lines (0.5 µm)

T: the movement time (sec)

We have calculated the speed of 10 spermatozoa, then we calculated the average speed

2-7-3-Sperm mobility

The number of sexually motile sperm was then estimated in duplicate directly check the reading under a light microscope at a magnification of at least 100X

The percentage of forward motile sperm is calculated using the following formula next :

$$M = \frac{\mathrm{T} - \mathrm{I}}{\mathrm{T}} \times 100$$

M: Mobility % (mobile sperm %)

T: total sperm count

I: immobile sperm count

2-7-4-Viability

Sperm viability assessed by staining eosin

The principle of eosin staining is that the dead sperm turn red, while the living remain transparent. Completely or partially stained pink sperm are considered as dead. On the contrary, those that appear colorless or with a very slight color limited to the midpiece area of the sperm are considered alive.

On a slide a drop of the diluted sperm and other drop of 0.1% eosin has been put instead of eosin 1% because the head of the sperm rats have an affinity for eosin at higher concentrations than human sperm, the drops were smeared and air-dried.

2-7-5-Sperm concentration

After carrying out the manipulation, the cell concentration of the cell suspension studied is calculated.

$$N = \frac{n}{a.v} \times F$$

n: number of cells counted

v: counting volume

F: dilution factor

N: number of cells per unit volume.

a: number of counted units.

3-Statistical analysis

The normality and Levene test are used to control the homogeneity of the variances of both treated and control group, when they are homogeneous, the mean comparison test (student test) is used if the P value is less than 5% ($P \leq 0.05$) a significant difference is found between the groups.

If the normality test was negative, the Mann-Whitney test is used, when the P value

($P \leq 0.05$) there is a significant difference.

All these operations carried out using SPSS software.

RESULTS AND DISCUSSION

1-Clinical signs and behavioural changes

During the treatment period we haven't recorded any signs of acute or chronic intoxication. In the first days we observe that the activity reduced and the rats sleeps after treatment in contrast with the control group this corresponds for its sedative and sleep enhancing effects according to **Chaitra, et *al*.,(2020)** study.

Also, we haven't observed any stress and behavioural changes neither signs of chronic or acute pain.

Photo 5 : Post treatment result.

2-Food Consumption

Descriptively there was a difference between the two groups in food intake.

Due to the following graphic curve in figure and according to the statistical analysis test.

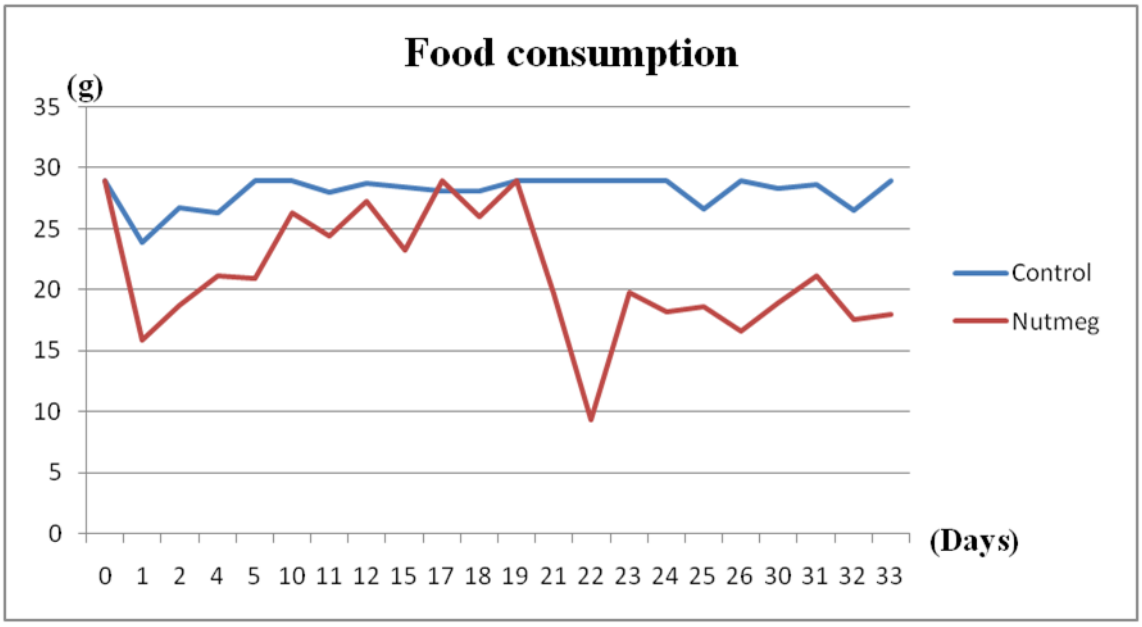

Figure 3 : Graphical curve representing food consumption (g) during the treatment period.

According to Mann Whitney test results, we observe that there was a significant difference ($P \leq 0.05$) between the control and treated group in the first days of treatment also there was a significant decrease in food consumption of the treated group maybe it's a result of nutmeg effectiveness on appetite or due to manipulation. From the 7^{th} to 12^{th} day there was a non-significant difference ($P \leq 0.05$) between the two groups, perhaps this is due to animal's adaptation to manipulation and treatment.

From the 13 days until the end of the treatment there was a significant difference ($P \leq 0.05$) in food intake between control and treated group this confirm the effectiveness of MFAE or its effect on the appetite of animals, which led to decrease in food intake.

3-Body Weight

As a result of the descriptive analysis to the body weight's evolution with treatment period of the two groups, we observe that the body weight of the group treated with MFAE decrease with duration of exposure in the opposite the other group (control) we observe an increase in the body weight of rats in the next graphic curve in figure.

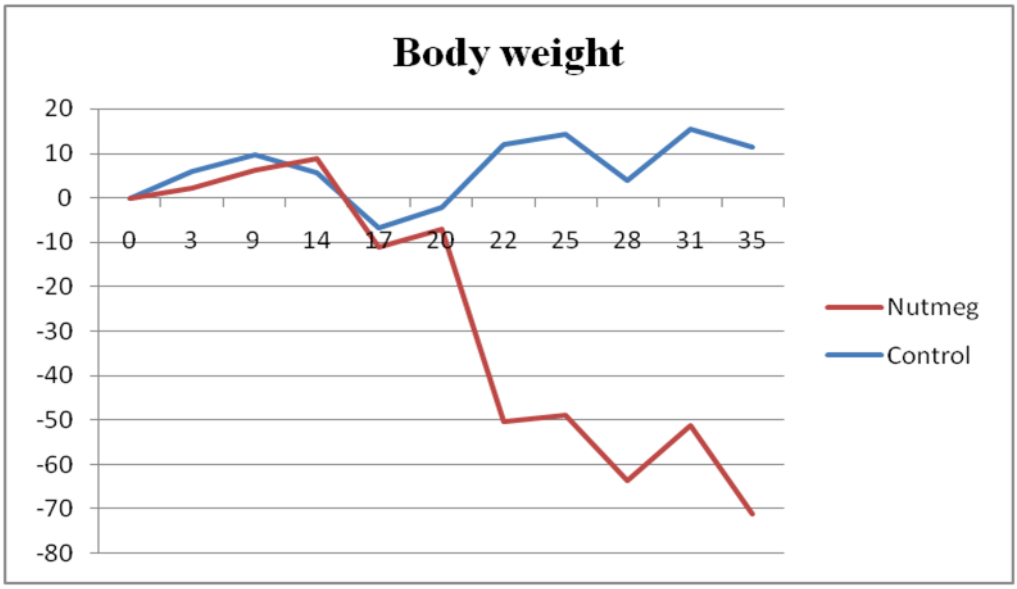

Figure 4: graphic curve representing the evolution of the difference between initial and final body weight during treatment period.

According to previous studies it was mentioned that nutmeg have anti-obesity effect **(Nguyen, *et al.*, 2010)**, that could explain the decrease in the body weight of the treated rats with MFAE. Since there is a decrease in food intake as result there is a decrease in body weight, which indicate the relation between them.

It was reported by **(Meera, *et al.*, 2009)** that oral treatment of *Myristica fragrans* oil did not cause any significant change in the body weight that was in contrast with our results, that maybe due to the difference in the chemical composition of the oil and MFAE.

4-The correlation between food intake and body weight

We suggest that the decrease in food intake in MFAE group cause a significant decrease in the body weight in contrast with the control group. After statistical analysis, the test proves that there's non-correlation between body weight and food intake. In this case, this confirms the direct effectiveness of MFAE on weight.

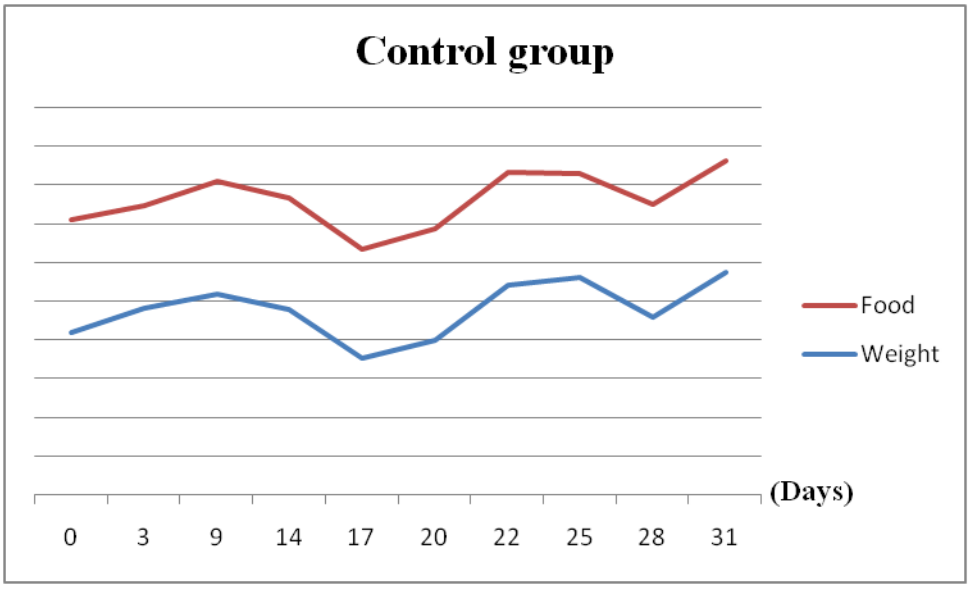

Figure 5 : A graphical curve represent the evolution of both food intake and body weight of the control group during the treatment period.

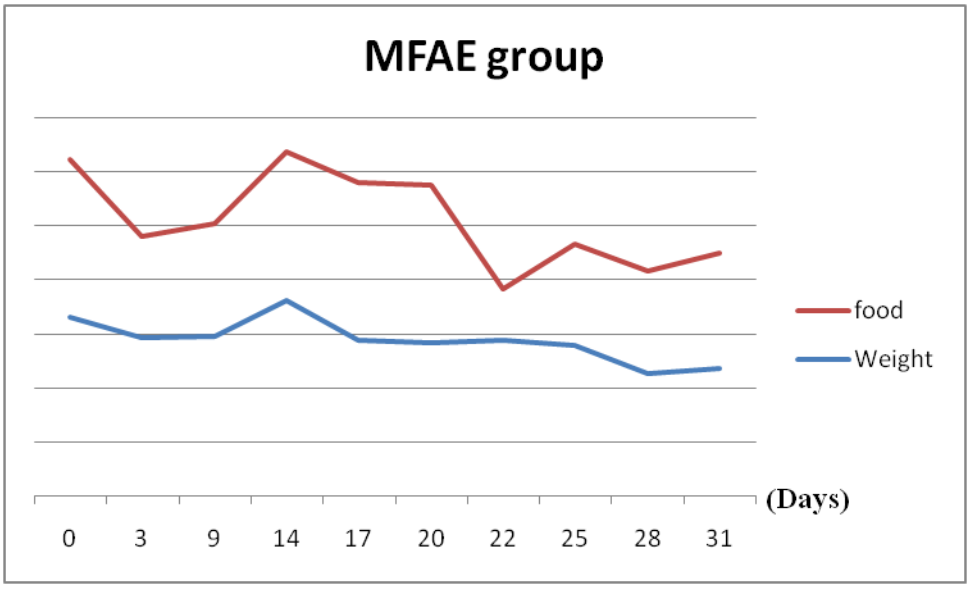

Figure 6 : A graphical curve represent the evolution of both food intake and body weight of the MFAE group during the treatment period.

5-Organ's weight

The descriptive analysis shows that there is a difference in the weight of the organs, an increase in the organs weight of the rat's treated with MFAE as compare to the control group was observed

According to the results of statistical analysis with Student test there was a significant difference ($P \leq 0.05$) in the weight of the organs between control and MFAE treated group.

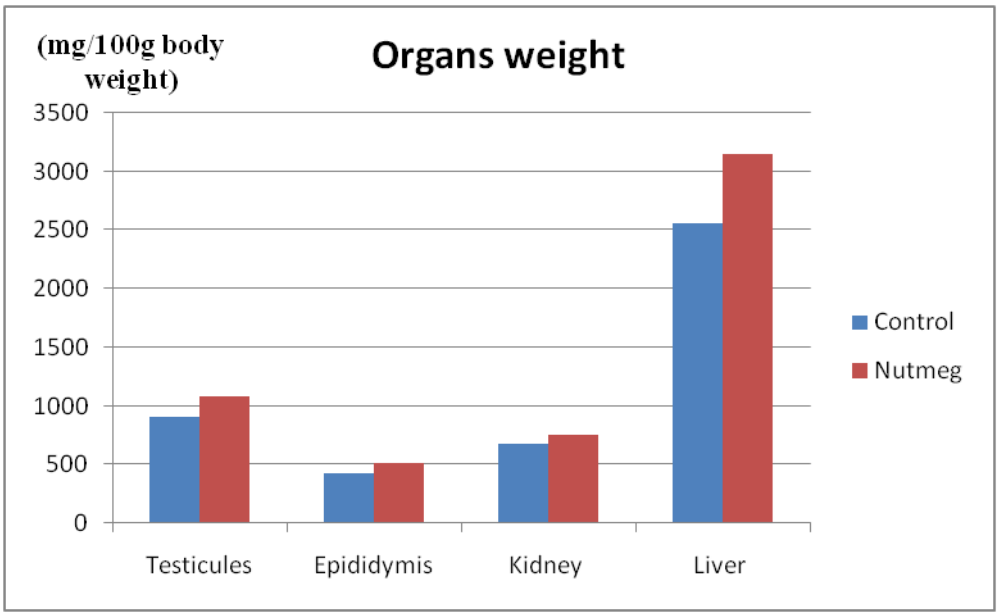

Figure 7 : Graphic columns representing the average of organs weight of both treated and control group (mg/100g body weight).

According to the results of the current study MFAE doesn't lead to a decrease in the weight of the testicles, and this contradicts the study of **Meera, et *al*.,(2009)**, which found that there was a significant decrease in the weight of testicles in rats treated with Myristica fragrans oil, this may be due to the difference in the chemical composition of the oil and MFAE.

Also, the same study by **Meera, et *al*.,(2009)**, found that was a significant decrease in the epididymis weight and non-Significant difference in the liver's weight, these results were in contrast with our results, both of the epididymis and liver weight increase.

There was a significant change in kidney's weight in both treated and control group.

Table 3 : Organs weight of control and treated group.

Tissue weight (mg/100g body weight)				
	Testicules	Epididymis	Kidney	Liver
Control	910,93±29.21	425,36±17.45	682,49±25.21	2554,47±91.13
Nutmeg	1083,67±26.78*	512,58±16.43*	758,73±17.69*	3154,74±147.7*

Mean ± SEM, * P≤0.05 (significant difference).

So MFAE make a significant difference in the tissue weight (mg/100g body weight)as compared to the control group there was an increase, that's may be due to the decrease in body weight.

6-Serum testosterone levels and biochemical analysis

In our results we found that there was a significant decrease in testosterone levels on the MFAE treated group, as compare to the control group the next graphic shows serum testosterone levels.

That was in agreement with **(Meera, *et al*., 2009)** results.

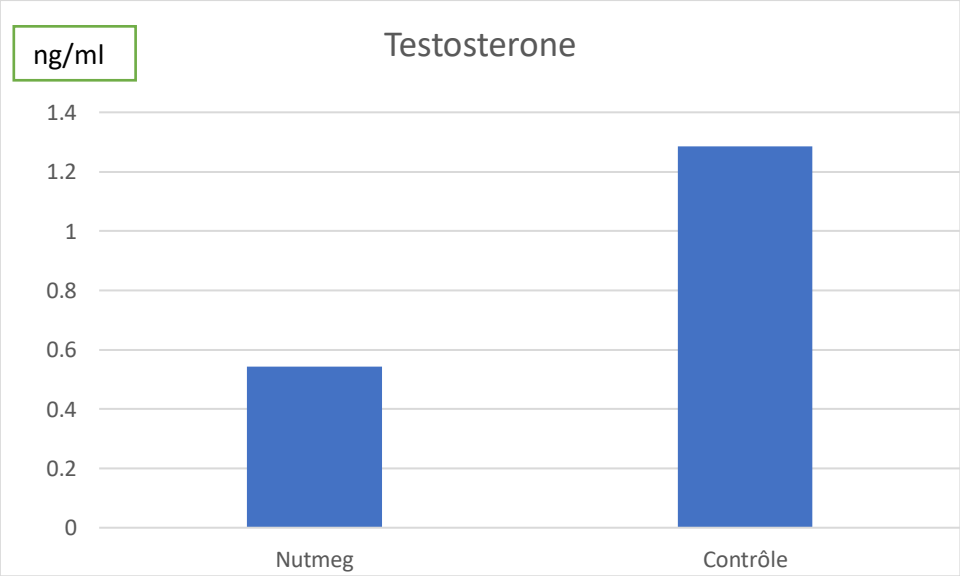

Figure 8 : Graphic columns representing the average of testosterone levels in the two groups.

According to biochemical analysis there was non-significant difference between the two groups.

Table 4 : Biochemical analysis results.

	Control	**MFAE**
Blood urea	0,35 g/L	0,39 g/L
Blood creatinine	5,18 mg/L	6,12 mg/L
Blood uric acid	10,5 mg/L	13,2 mg/L
TGO (ASAT)	96,21 UI/L	118,3 UI/L
TGP (ALAT)	51,55 UI/L	63,79 UI/L
PAL (alkaline phosphatase)	139 UI/L	208 UI/L

7-Sperm study

7-1-Concentration

According to previous study by **Hussein & Ayoub,. (2007)**, a dose of 250 and 500 mg/kg of nutmeg oil extract resulted in a significant decrease in sperm count that was in agreement with our results, after statistical analysis we found that there was a significant decrease in sperm count in rat's treated with MFAE as compare to the control group

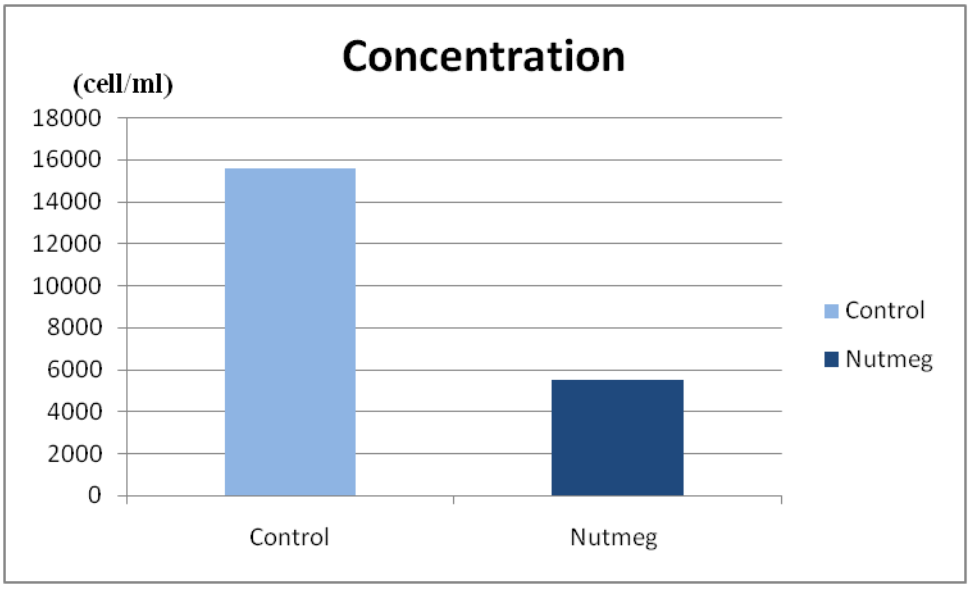

Figure 9 : Graphic columns represent the average of sperm concentration in the treated and the control group.

7-2-Mobility

There is significant difference between the two groups in sperm mobility percentage these results suggest that nutmeg have an effect on sperm mobility.

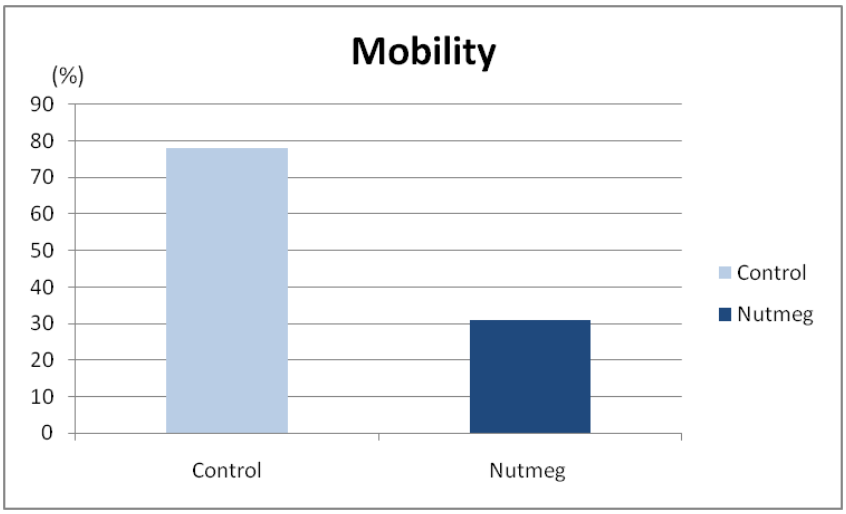

Figure 10 : Graphic columns represent the mean sperm mobility percentage of both treated and control group.

7-3-Velocity

According to statistical analysis there was a significant difference between the two groups in sperm velocity. It was observed that sperm velocity in rats treated with MFAE was higher than

The control group velocity

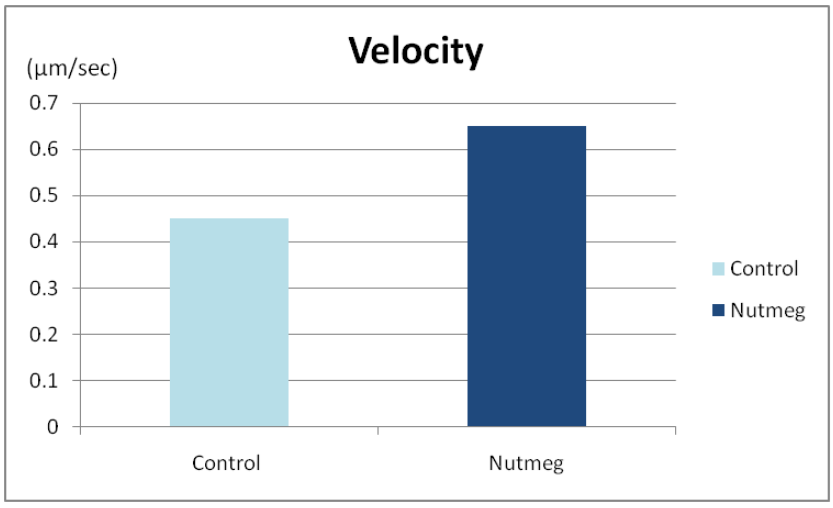

Figure 11 : Graphic columns represent the mean of sperm velocity (µm/sec) in the control and treated group.

CONCLUSION

Nutmeg extracts are a resource of many biologically active compounds that have therapeutic properties but they may also account for their toxicity from exposure to high doses.

After studying the effect of an aqueous extract of nutmeg seed in a dose 400 mg/kg body weight by oral administration for 33 days on some male fertility parameters, biochemical parameters, body weight evolution with the treatment period and organs weight we obtained a set of different results.

That results show changes in body weight, organs weight, testosterone levels, sperm count, velocity and mobility in addition to the absence of any change or effect on biochemical parameters , which proves the antifertility effects of nutmeg in males. Our study's results were somehow inconsistent with previous studies, and with regard to the results of the sperm study as well, where an increase in sperm velocity and a decrease in concentration and mobility were recorded, that makes us put a hypothesis that nutmeg has an effect on the spermatogenesis either in cell division (mitosis, meiosis) or on spermatozoa maturity This also may be a result of low serum testosterone levels, due to its important role in controlling spermatogenesis, and this also will be an explanation for the lack of influence on the speed, or rather its increase. Perhaps the nutmeg extract entered into this increase. We assume that MFAE have a positive effect on sperm velocity.

Regarding all these interesting results, it still needs to confirm with histological study with as well with larger number of animals to reduce the error rate.

As a conclusion the chronic exposure to *Myristica fragrans* aqueous extract may led to decline in male fertility in relation with dose and duration of exposure.

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Abstract

Myristica fragrans Houtt also called Nutmeg, has long been used to treat male sexual disorders. The aim of our current work is to study the chronic effect of an aqueous extract of nutmeg in a dose 400 mg/kg body weight on male *Albino Wistar* rats fertility.12 males used in the realization of this experiment, oral administration of the extract in a dose of 400 mg for 33 days and daily resulted in a decrease in the weight of the rats, a decrease in food intake, decreased levels of testosterone, also a decrease in sperm concentration and motility except for organs weight and sperm velocity there was a significant increase all this compared to the control group. As a conclusion, continuous exposure to nutmeg in specific doses and for long periods may lead to a decrease in male rats fertility.

Key words: *Myristica fragrans*, infertility, male rats, Antifertility effects

Résumé

Myristica fragrans Houtt également appelé la noix de Muscade, est utilisée depuis longtemps pour traiter les troubles sexuelle masculine. L'objectif de nos travaux actuels est d'étudier l'effet chronique d'un extrait aqueux de noix de muscade sur la fertilité des rats *Albinos Wistar* mâles albinos. 12 mâles utilisés dans la réalisation de cette expérience, l'administration orale de l'extrait à la dose de 400 mg pendant 33 jour et quotidiennement a entraîné une diminution du poids des rats, une diminution de la prise alimentaire, une diminution du taux de testostérone, également une diminution de la concentration et de la motilité des spermatozoïdes sauf pour le poids des organes et la vélocité des spermatozoïdes, il ya eu une augmentation significatif de tout cela par rapport au groupe témoin. En conclusion, une exposition continue à la noix de muscade à des doses spécifiques et pendant de longues périodes peut entraîner une diminution de la fertilité des rats male.

Mots clés : *Myristica fragrans*, infertilité, rats males, effets antifertilité.

الملخص

لطالما استخدمت جوزة الطيب لعلاج الاضطرابات الجنسية للذكور في الطب البديل ،الهدف من دراستنا الحالية هو دراسة التأثير المزمن لمستخلص مائي لبذور جوزة الطيب على خصوبة جرذان من نوع *Albinos Wistar* ، تم استخدام 12 ذكر لتحقيق هذه التجربة. أدى تناول المستخلص عن طريق الفم بجرعة 400 مجم لمدة 33 يومًا ويومياً إلى انخفاض في وزن الفئران ، وانخفاض في تناول الطعام ، وانخفاض في معدل هرمون التستوستيرون ، وكذلك انخفاض في تركيز الحيوانات المنوية وحركتها باستثناء وزن الأعضاء وسرعة الحيوانات المنوية ، كان هناك زيادة معنوية في كل هذا مقارنة بمجموعة التحكم. في الختام ، يمكن أن يؤدي التعرض المستمر لجوزة الطيب بجرعات محددة ولفترات طويلة إلى انخفاض الخصوبة في ذكور الجرذان.

الكلمات المفتاحية: جوزة الطيب ، العقم، ذكور الجرذان، تأثيرات مضادة للخصوبة