

Effect of nut meg (*Myristica fragrans*) oil extract on testicular function in mice exposed to oxidative stress.*

Abdul kareem Hussain Ismail
physiology, College of Education

Rafah sami Ayoub
College of Dentistry

University of Mosul

الخلاصة

ذكرت جوزة الطيب في الطب اليوناني لما لها من فائدة في علاج الاضطرابات الجنسية. الدراسة الحالية تقيم تأثير المستخلص الزيتي لجوزة الطيب على وظائف الخصية في الفئران المعرضة إلى 1% بيروكسيد الهيدروجين ولمدة 21 يوماً. تم إعطاء 250 ملغم/كغم من وزن الجسم من المستخلص الزيتي لجوزة الطيب عن طريق الفم للفئران الذي أدى إلى انخفاض معنوي في وزن الخصية وعدد النطف والنسبة المئوية للنطف الحية والطبيعية عند مقارنتها مع المجموعة المعرضة لبيروكسيد الهيدروجين. أما عند إعطاء جرعة 500 ملغم/كغم من وزن الجسم من المستخلص الزيتي لجوزة الطيب أدى إلى انخفاض أكثر في وزن الخصية وعدد النطف والنسبة المئوية للنطف الحية والطبيعية وبشكل معنوي عند مقارنتها مع المجموعة المعرضة لبيروكسيد الهيدروجين وأدى إلى قلة عدد الخلايا الجرثومية الذكرية بشكل معنوي عند مقارنتها مع المجموعة المعرضة لبيروكسيد الهيدروجين والسيطرة. وتظهر نتائج الدراسة الحالية استعمال جوزة الطيب بشكل مرغوب في الطعام لاحتوائه على بعض المواد المضادة للأكسدة لكن ليس بالجرع العالية حيث أن جرعة 250 و 500 ملغم/كغم من وزن الجسم تعتبر سامة للخصية .

Abstract:

nut meg (*Myristica fragrans*) has been mentioned in ancient medicine to be beneficial in the managment of male sexual disorders. The present study is to evaluate the nut meg oil extract on testicular functions in mice exposed to 1% hydrogen peroxide for 21 days. Oral administration of nut meg oil extract at adose of 250 mg/kg produced asignificant decrease in testis weight and sperm count and percentage of viable and normal sperm as compared to hydrogen peroxide treated group. Oral administration of nut meg 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

*Presented at the first conference on Biology , University of Mosul ,college of Education , 4-5 September 2007

compared to hydrogen peroxide treated group and control. The present results suggest that nut meg popularly consumed as food and may contain some active principles with anti oxidant properties. However high doses of 250 or 500 mg/kg could be very toxic to the testis.

Introduction:

Nut meg (*Myristica fragrans*) is widely used as flavour spice. Many kinds of baked goods, sausages and beverages (1) and is used in alternative medicine as an anti-diarrheal agent for patients with medullar carcinoma of thyroid gland. The effect of the treatment may be due to inhibition of prostaglandin synthesis in the mucosa and sub mucosa of the colon, it is used some times as stomachic, stimulant, carminative, to control flatulence, and has areputation as an emmenagogue and abortifacient (1,2). It has also been reported to have antioxidant property (3). Photochemical studies indicate that nut meg contains a volatile oil, fixed oil, proteins, fats, starch and mucilage. The fixed oil contains myristicin and. Myristic acid. Nut meg yields (5-15%) of volatile oil which contains pinene, sapinene, camphene, myristicin, elemicin, iso elemicin, eugenol, isoeugenol, methoxyeugenol, safrol, dimeric phenyl propanoids, lignase and neolignase (4,5). Eugenol the major constituent inhibits lipid peroxidation and maintains activities of enzymes like superoxide dismutase, catalase glutathione peroxidase, glutamine transferase and glucose 6-phosphate dehydrogenase (6). It was observed that seed extract of *M.fragrans* showed significant calcium channel antagonistic activity (7). And has hepato protective effect on lipopoly saccharide/D-Galactosamine induced liver injury (8). Both nutmeg crude suspension and petroleum ether showed a good anti diarrheal effect with significant sedative property. The extracts possessed only a weak analgesic effect with no harmful effects on blood pressure & ECG. (9). It has also been found that 50% of ethanolic extract of *M.fragrans* and *syzygium aromaticum* (L) Merr, and perry (clove) have aphrodisiac activity by stimulating the mounting behavior of male mice and significantly increased their mating performance (10), also 50% of ethanolic extract of nutmeg alone induced aphrodisiac activity in rats by increasing lipido and potency (11). *M.fragrans* n-hexane has been reported to have memory enhancing effect in mice (12). The present work is designed to investigate the effect of nut meg oil extract on testicular function in mice exposed to hydrogen peroxide as an oxidative stress inducer, then evaluate whether the nutmeg oil extract could decrease or increase testicular function and spermatogenesis in male mature mice.

Materials and Methods:

Plant material: *Myristica fragrans* seeds were purchased from local market of Mosul, Iraq, it was identified by a plant taxonomist, Department of biology faculty of science. University of Mosul.

Preparation of extract: The oil of *M. Fragrans* was prepared in this study according to Taga (13) by the pressed 5Kil0/neuton to 100 gram of dry clean plant to obtain 6.2 gram of plant oil.

Animals: Adult healthy swiss male mice, 25-30 gm were used in this experiment which were housed in plastic cages under standared conditions of light and darkness 12:12 with ambient temperature 20 ± 2 . C . They were fed with standared labratory chow and water ad libitum.

Protocol design:

Group I: served as control received tap water .

Group II: received 1% H₂O₂ (hydrogen peroxide) with drinking water for 21 days.

Group III: exposed to 1% hydrogen peroxide with drinking water and treated at the same time with 250 mg/kg of body weight oil extract of nutmeg for 21 days by oral intubation.

Group IV : exposed to 1% hydrogen peroxide with drinking water and treated at the same time with 500 mg/kg of body weight oil extract of nutmeg for 21 days by oral intubation.

The dose of nut meg oil was given according to Tajuddin (10). Daily observations were done all animals at the end of the experiment the mice were weighed and killed by using an over dose of ether. Testis and epididymis were trimmed of fat prior to recording their weight of both sides. After weighing testis epididymis of each animal was dissected, cleared and epididymal sperm was collected by squeezing caudal region of the epididymis after cutting it in to pieces , according to Sorensen(15), the semen sample is diluted 1:10 the diluent solution was 5% formal bicarbonate. The count of spermatozoa was determined using haemocytometer Method also assessment of the percentage of live and dead sperms (viability) according to Sorensen (15) live and dead sperms were distinguished by adding one drop of Eosin-necrosin stain to one drop of semen at room temperature then the mixture is smeared on slide and examined at (40X) power. One hundred spermatozoa were classified as either colored if the stain has passed through the membrane, the cell in considered dead or non stained and the cell considered alive , also assessment of the sperm morphology were evaluated as follows, normal, head defect, middle piece defect, principle piece defect, proximal droplet, distal droplet and detached head. In sperm with two or more defects only. The defect afflicting its more proximal region was recorded (15).

Histopathology of the testis:

The testis were removed and weighed then kept in formal saline solution for histological studies. Fixed testis were sectioned at 6Mm mounted and stained with H&E. fifty seminiferous tubules at the same stage were analyzed for each group. The mean diameter of seminiferous tubules was measured using ocular micrometer. The mean number of spermatogonia, primary spermatocytes and spermatids per tubular cross section were counted.

Statistical analysis:

Data were analyzed using one way analysis of variance (ANOVA). Groups difference were determined using Duncans multiple Range test. The results were expressed as mean \pm S.E.M ($p < 0.05$) was considered statistically significant (16).

Results:

Table (1) shows the weight of testis and sperm count and percentage of viable and normal sperm in different animal groups. Mice treated with 1% hydrogen peroxide shows a significant decrease ($p < 0.05$) in sperm count and percentage of viable and normal sperm when compared with control while the weight of testis did not show significant differences as compared with the control. 250 mg/kg oil extract of nutmeg and 1% hydrogen peroxide administered to mice for 21 days at the same time caused a significant ($p < 0.05$) decrease in weight of testis and sperm count and percentage of viable and normal sperms as compared to 1% hydrogen peroxide treated group and control. 500 mg/kg oil extract of nutmeg and 1% hydrogen peroxide administered to mice for 21 days at the same time caused a significant ($p < 0.05$) decrease in testis weight and sperm count and percentage of viable and normal sperms compared with 1% hydrogen peroxide group and the control. Yet produced worse deterioration than nut meg at (250 mg/kg).

Table (2) shows the mean diameter of seminiferous tubules and mean number of spermatogonia and primary spermatocyte and spermatid per tubular cross section. 1% hydrogen peroxide administered for 21 days resulted in a significant ($p < 0.05$) decrease in mean number of spermatogonia and primary spermatocyte and spermatid per tubular cross section compared with the control. 250 mg/kg of oil extract of nutmeg and 1% hydrogen peroxide administered to mice for 21 days at the same time produced no significant changes in mean diameter of seminiferous tubule & mean number of spermatogonia and primary spermatocyte and spermatid while 500 mg/kg of oil extract of nutmeg and 1% hydrogen peroxide to mice for 21 days at the same time gave a significant ($p < 0.05$) decrease in count of spermatogonia compared with 1% hydrogen peroxide treated group and the control. Yet the count of primary spermatocyte and spermatid decrease more than the dose of 250mg/kg oil extract of nutmeg.

Table (1). Effect of nutmeg oil extract on weight of testis and sperm count and percentage of viable sperm and normal sperm in adult male mice treated with 1% hydrogen peroxide

Experimental groups	Weight of testis mg/100gm body weight	Sperm count $\times 10^6$	Percentage of viable sperm	Percentage of normal sperm
Control	B 440 \pm 0.07	D 32.11 \pm 0.85	D 79.11 \pm 1.5	D 85.19 \pm 1.1
1% H2O2 for (21) days	B 420 \pm 0.08	C 20.23 \pm 0.62	C 58.22 \pm 1.3	C 43.32 \pm 0.92
1% H2O2 + <i>M. fragrans</i> 250 mg/kg oil extract for 21 days	A 380 \pm 0.03	B 18.20 \pm 0.82	B 52.52 \pm 0.9	B 38.21 \pm 1.2
1% H2O2 + <i>M. fragrans</i> 500 mg/kg for 21 days	A 350 \pm 0.04	A 15.00 \pm 0.88	A 40.34 \pm 1.1	A 33.31 \pm 1.7

Values are mean \pm S.E.M

Different letters are significant at $p < 0.05$

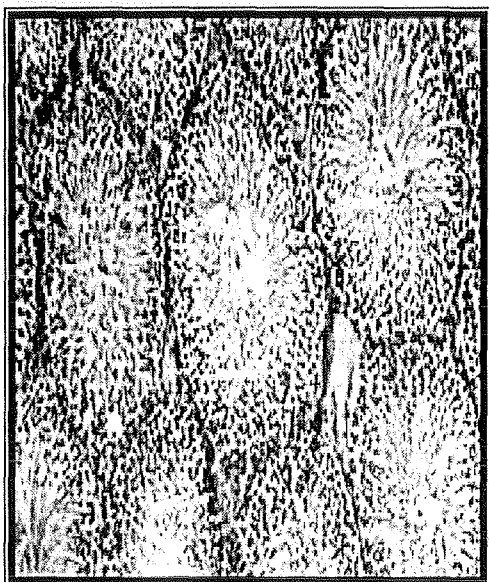
Table (2). Effect of nutmeg oil extract on the mean diameter of seminiferous tubule, mean number of spermatogonia, primary spermatocytes and spermatid per tubular cross section in male mice treated with 1% hydrogen peroxide

Values are mean \pm S.E.M

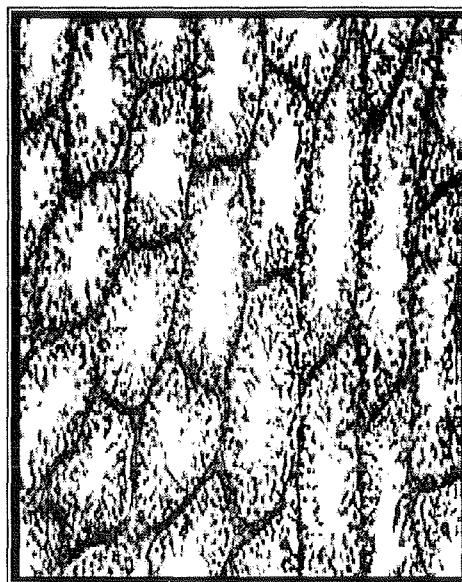
Experimental groups	mean diameter of seminiferous tubule (Mm)	mean no. of spermatogonia / tubular cross section	Mean no. of primary spermatocytes / tubular cross section	mean no. of spermatid / tubular cross section
Control	A 120.09 \pm 2.7	C 33.5 \pm 1.41	B 45.0 \pm 5.6	B 20 \pm 2.7
1% H2O2 for 21 days	A 123.70 \pm 1.53	B 30.5 \pm 2.6	A 20.9 \pm 5.7	A 8 \pm 1.5
1% H2O2 + <i>M. fragrans</i> 250 mg/kg oil extract for 21 days	A 122.10 \pm 2.67	B 27.5 \pm 1.41	A 15.5 \pm 4.2	A 5 \pm 2.0
1% H2O2 + <i>M. fragrans</i> (500 mg/kg) for (21) days	A 121.10 \pm 1.67	A 18.7 \pm 2.6	A 13.3 \pm 5.5	A 3.3 \pm 1.2

Values are mean \pm S.E.M

Different letters are significant at $p < 0.05$



Fig(1) Section in mice testis of control group showed sertoli cell II. Spermatocytes III. spermatozoa



Fig(2) Section in testis of mice administrated with 1% H_2O_2 showed necrosis of most spermatogenic cells



Fig(3) Section in mice testis administrated 1% H_2O_2 + 250 mg/kg *Nut meg* showed necrosis of all spermatogenic cells



Fig(4) Section in mice testis which administrated 500mg/kg of + 1% H_2O_2 Nut meg note the necrosis of most spermatogenic cells and disappearances of spermatoc

Discussion:

Results of the present study showed that administration of 1% H₂O₂ for 21 days to mice caused a significant decrease in sperm count and percentage of viable and normal sperm and count of spermatogonia and primary spermatocyte and spermatid. These results were in agreement with Ismael (18) and Aziz (17) these may be attributed to reactive oxygen species (ROS) which result in peroxidative damage to the cell lipid membrane. Poly unsaturated fatty acids are the most susceptible to lipid peroxidation presumably, because of the presence of carbon-carbon double bonds that weaken the carbon-hydrogen bond on the adjacent hydrogen atom that make it susceptible to cleavage. Lipid peroxidation triggers the loss of membrane integrity, causing increased cell permeability to electrolytes. The inward leakage of calcium and sodium ions in particular can affect the cell to become -ATP- depleted, and the increase in intracellular calcium ions also activate protease and phospholipase which can lead to further damage to proteins and lipids. This free radical mediated process may also cause enzyme inactivation with structured damage to DNA which causes cell death (19-21).

A dose of 250 and 500 mg/kg of nutmeg oil extract resulted in a significant decrease in testis weight sperm count and percentage of viable and normal sperm. The Histological investigations revealed a progressive increase in degeneration of seminiferous epithelial cells of the testis and these results were in agreement with Olaleye(3) in rats. The results suggests that the consumption of large quantities of this extract causes low sperm count & affect the sperm viability and integrity and affect the germinal epithelium, although it has been reported to be capable of improving sexual activity (10,11).

Nut meg oil extract produced no protective effect in this study due to the fact, that degenerative changes occurred in germinal epithelium of testis were irreversible.

In conclusion: the reported findings demonstrated that secondary metabolites which are largely responsible for therapeutic or pharmacological activities of medicinal plant perry (22) , may also account for their toxicity when the dosage is abused. Caution should therefore be advocated in the intakes of this product.

References:

- 1- Panayotopoulos DJ, Chisholm DD. Hallucenogenic effect of Nutmeg. British medical J.,1, 754-760(1970).
- 2- Green Rc. Nutmeg poisoning J. Jama 177,1342-1344, (1959).
- 3- Olaleye MT, Akinmoladun Ac, Akindahunsi AA. Antioxidant properties of *Myristica fragrans* (Houtt) and its effect on selected organs of albino rats. African J. of Biotechnology, (2006), 5, 1274-1278.
- 4- Isogai A, Suzuki A, Tamura S.. Structure of dimeric phenoxypropanoic from *Myristica fragrans*. Agar Biol chem(.,1973), 37,193-194.
- 5- Janssen J, Laeckman GM. Nutmeg oil: Identification and quantification of its most active of platelet constituents as inhibitor aggregation J Ethnopharmacol., (1990), 29:179-188.
- 6- Kumara velu SS, Dakshin murthy DP, Devra NS. The antioxdant effect of eugenol on carbon-tetrachloride-induced erythrocyte damage in rats. J Nut Biochem., (1996),7, 23 – 28.
- 7- Muhammad J, Fagir F, Qureshi H, Malik SA, Muhammad MA. Evaluationof effects of Extract from seeds of *M. fragrans* on volume and acidity of stimulated gastric secretion, liver and kidney function. J P M I (Journal of post graduate medical institute), (2004) 18, 644-650.
- 8- Morita T, Jinno K, Kawagishi H, Arimota Y, suganuma H, Inakuma T and sugiyama K., Hepato protective effect of myristicin from nutmeg (*M-fragram*) on lipopoly saccharinde /D-galactos amine include liver injury. J. Agric. Food chem. (2003), 51, 1560-1565.
- 9- Grover JK, Khandkar S,vats V, Dhunoo Y, Das D pharmacological studies on *Myristica fragrans* anti diarrheal , hypnotic , analgesic and hemodynamic (blood pressure) parameters. Methods find EXP clin pharmacol . (2002).24, 675-680.
- 10- Tajuddin As , latif A , Qasmi /A.Aphrodisiac activity of 50% efhanolic extracts of *M.fragrans* Houtt (nut meg) and *syzygium aromaticum* (L) merr.and perry (clove) in male mice, acomparative study (BMC) complement Altrernative Med. (2003), 3, 6.
- 11- Tajuddin As, Shamshad A, Latif A, Iqbal A, Qasmi and Yusufamin KM. An experimental study of sexual function improving effect of *Myristica fragrans* Houtt (nutmeg). BMC. Complementary and alternative medicine, (2005),5, 16.
- 12- Parle M, Dhingra D, Kulkarni S:, improvement of mouse memory by *Myristica fragrans* seeds J. Med food, (2004),7,157-161.
- 13- Taqa AA, Taqa QA, Al-Hamadani IH. The effect of oil piper cubeba on the cariogenic bacteria (strepto coccus mutans and staphylococcus). Mustansiria dental Journal, (2005),2, 92-95.

- 14- Hassan AB, Ayoub MM, Doghaim RE, Yousef HI, Eid AA. Studies of the effect of some antihistamines on the reproductive organs of male rat. *Vet. Med. J.* (1983), 31, 277-286.
 - 15- Sorensen AM. *Animal reproduction principle and practice*. McGraw-Hill book company Baltimore (1979), PP. 428-431.
 - 16- Steel RG, Torrie JH: *principle and procedures of statistics*. McGraw-Hill book, Inc. Newyork. (1993), PP 60-120.
 - 17- Aziz BN,. Effects of hydrogen peroxide-induced oxidative stress on epididymal sperms of mice. *Iraqi J. Vet. Sci.*, (2000), 13, 61-65.
 - 18- Ismael AH (2005). Protective effect of *Nigella sativa* oil on reproductive system and some blood parameters of Albino mice exposed to oxidative stress by hydrogen peroxide. Ph.D thesis college of Education, university of Mosul. Mosul. Iraq.
 - 19- Halliwell. B. Free radicals, antioxidants, and human disease: curiosity, cause or consequence? *Lancet*, (1994), 344, 721-724.
 - 20- Cummins JM, Jequier AM, kan R. Dithiotheitol effects on human sperm quality *J. Urol.*, (1994) 152, 2287-2290.
 - 21- Aitken RJ and clarkson, JS. cellular basis of defective sperm function and its association with the genesis of reactive oxygen species by human spermatozoa. *J. Reprod. Fertil*, (1987), 81, 459-496.
- Perry LM (1980). *Medicinal plants of East and south East Asia*, MIT press, cambridge Massachusetts.