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Toxic effects of water/alcoholic extract of *Syzygium aromaticum* on sperm quality, sex hormones and reproductive tissues in male mouse

Farzaneh Dehghani[1], Aseyeh Heshmatpour[2], Mohammad Reza Panjehshahin[3], Tahereh Talaei-Khozani[4*],

[1]*Histomorphometry & Stereology Research Center , Anatomy Department, Shiraz University of Medical Sciences (SUMS)*
[2]*Faculty of Pharmaceutics, SUMS*
[3]*Mohammad Reza Panjehshahin, Medical and Natural Product Chemistry Research Center, Pharmacology department, SUMS*
[4]*Anatomy Department, SUMS*

Abstract

Syzygium aromaticum was considered as an aphrodisiac agent; however, it may cause some side effects. The aim of this study was to examine the effects of *Syzygium aromaticum* extract on male reproductive system. To do this, forty male mice were divided into 5 groups: negative control was fed with vehicle, positive control was fed with aphridite and three experimental groups were fed with 250, 500 and 1000 mg/kg/day of water/alcohol extract of *S. aromaticum* for 34 days. The sperm count and motility and also the sex hormones level were assessed. The reproductive tissues were prepared histologically and studied under the light microscope. The results indicated the high dose-treated animals showed a significant decline in sperm count, motility and testosterone but a significant increase in estradiol concentration compared with the control group. The seminiferous tubules of extract-treated animals contained fewer sperms than in those of control animals. It seems that in spite of aphrodiastic activity of the *Syzygium aromaticum* extract, it reduced spermatogenesis.

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***Corresponding author**: Tahereh Talaei-Khozani (e-mail: t_talaee@yahoo.com)
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Introduction

Syzygium aromaticum or clove is a spice that has been used in herbal medicine since long time in Asia and Middle East countries. *Syzygium aromaticum* has also been used as remedy in herbal medicine. Raazi (926 A.D.) and Ive-e-Sina (1038 A.D.), two famous Iranian physicians, described the properties of *S. aromaticum* in medicine. A World Health Organization survey indicated that about 70–80% of the world population rely on non-conventional medicine, mainly of herbal sources, in their primary healthcare (Chan 2003). It is therefore important to study the side effects of the herbs used in traditional medicine to establish their effectiveness.

Syzygium aromaticum acts as anti-fungal (Pinto et al. 2009), anti-inflammatory and anti-microbial (Chaieb et al. 2007), immunemodulator (Carrasco et al. 2009), anti-carcinogenic (Zheng and Kenney 1992) and anti-mutagenic (Tajuddin 2003). This plant is an aphrodisiac agent and is used to cure sexual disorders in males (Buch et al. 1998). It has been shown that *S. aromaticum* oil was spermicidal on ejaculated human sperm (Mishra and Singh

2008). *Syzygium aromaticum* has been shown to increase sex hormone levels and active spermatogenesis in low doses, however, loosening of germinal epithelium or intraepithelial vacuolation were also revealed in some seminiferous tubules. The high dose of the extract was shown to be toxic to spermatogenesis (Chaieb et al. 2007).

The chemical composition of *S. aromaticum* was also investigated. Phenylpropanoids such as carvacrol, thymol and cinnamaldehyde (Nassar 2006), flavonoid triglycosides (Rastogi and Mehrotra 1984), eugenol, eugenol acetate, caryophyllene, sesquetrepene ester (Nassar 2006, Miyazawa and Hisama 2003) were found in *S. aromaticum* extract. Flavonoids act as agonist or antagonist of estrogen (Miyazawa and Hisama 2003, Miksicek 1995, Lucki and Sewer 2011) that may interrupt the hormonal system of the body and led to a decline in spermatogenesis. Among these, Eugenol has antioxidant activity (Abdel-Wahhab and Aly 2005, Gulcin 2011). Oxidative stress is the etiology of some kinds of infertility and impairs sperm motility, viability and morphology (Micinski et al. 2011). Antioxidants can improve sperm parameters and protect sperms from some kinds of cytotoxic components (Ben Abdallah et al. 2012); however, eugenol may show spermicidal activity (Carrasco et al. 2009).

Antioxidant and estrogenic properties of *Syzygium aromaticum* may influence the sperm quality, sex hormones levels and the integrity of reproductive tissues. The present study was designed to verify the side effects of *Syzygium aromaticum*.

Materials and methods

Extract preparation

Syzygium aromaticum were obtained from the local market, verified by a botanist. The herb was powdered to obtain water/ alcohol extract. Fifty g of powder suspended in 250mL petroleum ether for 2 h. The suspension was then filtered to separate the petroleum ether. The powder was then re-suspended in 300 mL 70% ethanol and the suspension was percolated. The ethanol was evaporated in a dessicator. The final yield was 15.8 g crystalline extract.

Animals and treatments

Forty male mice (BALB/c) weighted 25-30 g were divided into 5 groups of 8 animals each: 3 experimental groups and 2 control groups. The animals were kept under the standard conditions (12 light, 12 darkness and 23±2°C) ad labitum. The animals handling was in accordance to the guidelines of the ethic committee of the University. At the beginning of the experiment, blood samples were taken from the tail veins of the mice and the sera were collected and frozen in -20°C until used. The mice were then gavaged with 250, 500 and 1000 mg/kg/day of *S. aromaticum* extract for 34 days. The control groups were fed with the same volume of vehicle (as negative control) and aphrodit (as positive control) for the same period of time. On day 34, the mice were killed under deep anesthesia, blood samples were taken from their hearts and sera were collected and kept until used.

Sperm collection

On day 34, a transverse suture was made above the testes level under deep anesthesia and testes and ductus deferens were exposed. A transverse suture was made on the caudal part of ductus deferens and 1.5µL of semen was suctioned by a catheter with internal diameter 0.015mm linked to a syringe. The semen sample was diluted in 1 mL of normal saline. A hemocytometer was used for sperm counting. Sperm motility was assessed according to WHO criteria (World Health Organization 1999). The percentages of straight forward, slow progressive and immotile sperms were calculated.

Histopathological study

Testis, ductus deferens, epididymis, prostate and seminal vesicle were removed and fixed in 10% formalin. The tissues were prepared histologically, sectioned at 5µ thickness and stained with hemaoxylin and eosin. The specimens were examined with light microscopy

Hormone assay

The sera were assessed for the concentrations of testosterone and estradiol by immunoradioassay by a kit (Spectra, Finland) according to manufacturer's instruction.

Statistical analyses

The data were analyzed by Analyses of Variance (ANOVA) and independent T test. All statistical analyses were done by SPSS 13.0 software for Windows. A P-value less than 0.05 was considered as significant difference. All data were graphed with Excel.

Results

Hormone assay

The blood sex hormones of the experimental groups were compared with the control groups. The animals were fed with 500 and 1000 mg/kg/day of *S. aromaticum* extract and showed a significant decrease in testosterone level compared with the negative control ($P<0.05$). Aphridite (positive control) also led to a significant increase in testosterone concentration in serum compared with all experimental groups and the negative control ($P<0.05$). Estradiol concentration in serum just increased in the animals that were fed with 1000 mg/kg/day compared with the negative control group ($P<0.05$). Table 1 summarized the concentration of testosterone and estradiol in sera of the experimental, negative and positive control groups.

Table 1. Mean value ± SD of sera testosterone and estradiol concentrations in the *S. aromaticum* extract-treated mice and the controls mice

Groups	Testosterone	Estradiol
250 mg/kg	3 ± 1.323	7.5 ± 1.685
500 mg/kg	1.82 ± 0.928 *	8.72 ± 1.847
1000 mg/kg	1.26 ± 0.826 *	9.74 ± 1.506*
Negative control (vehicle)	3.93 ± 1.132	6.3 ± 1.223
Positive control (aphridite)	6.87 ± 1.716 †	6 ± 1.282

*Significant difference with the negative control group ($P<0.05$).

† Significant difference with all other groups ($P<0.05$).

Sperm quality

As depicted in figure 1, the administration of 1000 mg/kg/day of *S. aromaticum* extract led to a significant decrease in the sperm count ($P<0.05$), while the animals fed with aphredite had a significant higher number of sperm compared with the experimental groups and also the negative control groups ($P<0.05$).

The semen analysis of the animals treated with 1000 mg/kg/day showed a significant decrease in the percentage of the sperms with straight forward movement compared with the negative control animals ($P<0.05$). The percentage of the sperms with straight forward movement increased in positive control compared with the negative control groups ($P<0.05$). However, the extract did not change the percentages of the slow movement and immotile sperms ($P<0.05$) (Fig 2).

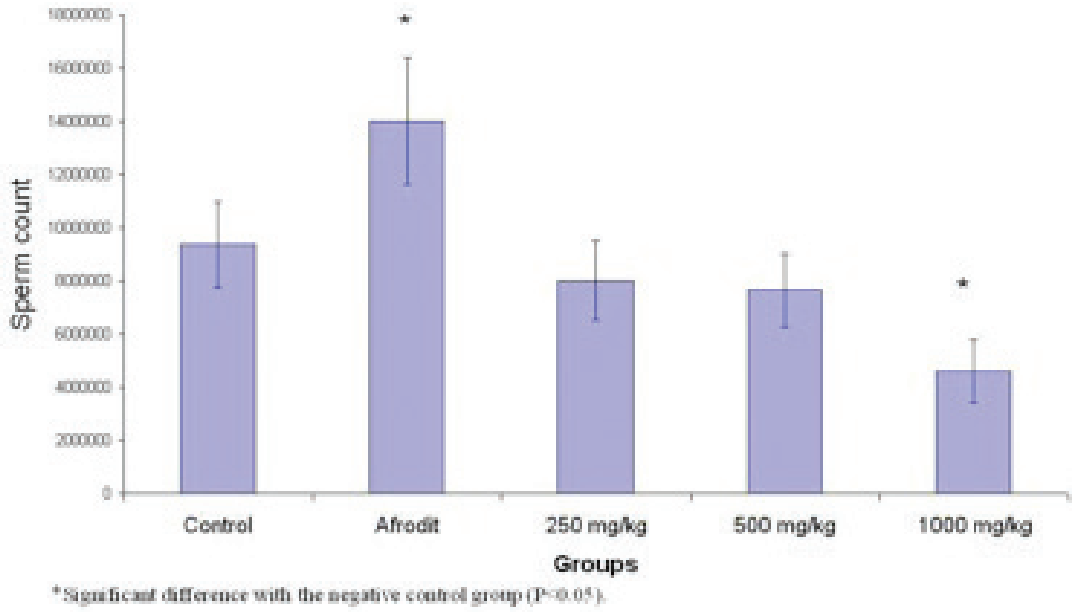

Figure 1. Sperm count in mice treated with *S. aromaticum* extract, aphridite and vehicle.

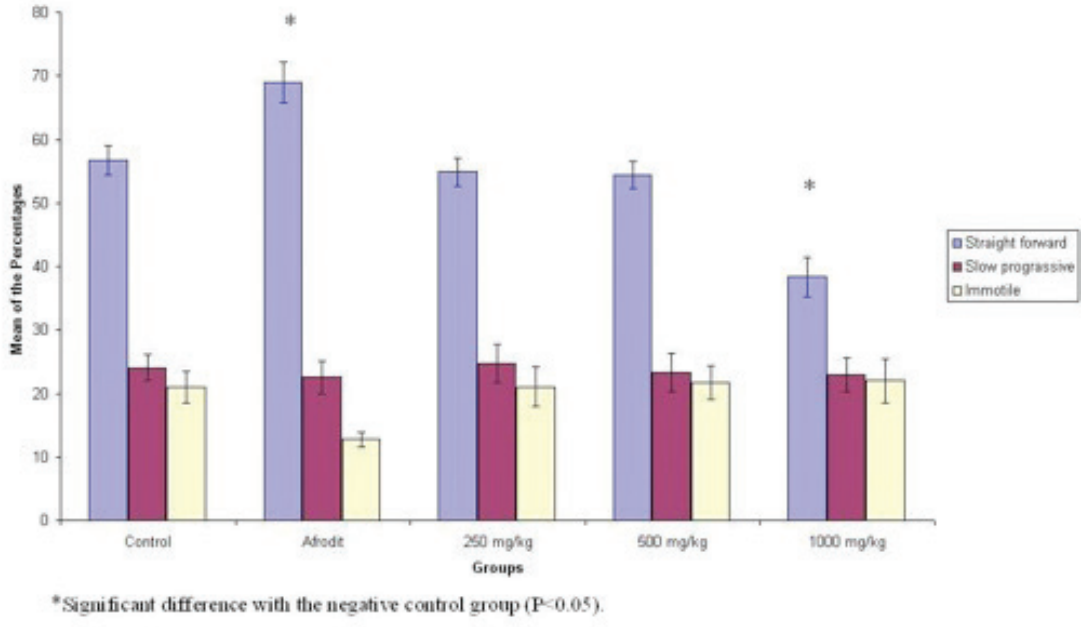

Figure 2. The percentage of motile sperm in mice treated with *S. aromaticum* extract, aphridite and vehicle.

Histopathological study

The seminiferous tubules in the testes of animals that were fed with 1000 mg/kg/day contained fewer sperms compared with those of the negative control animals (P<0.05). However, there was no difference in Leydig and Sertoli cell density in the testes of extract-treated animals compared with the negative and positive control groups (Fig 3).

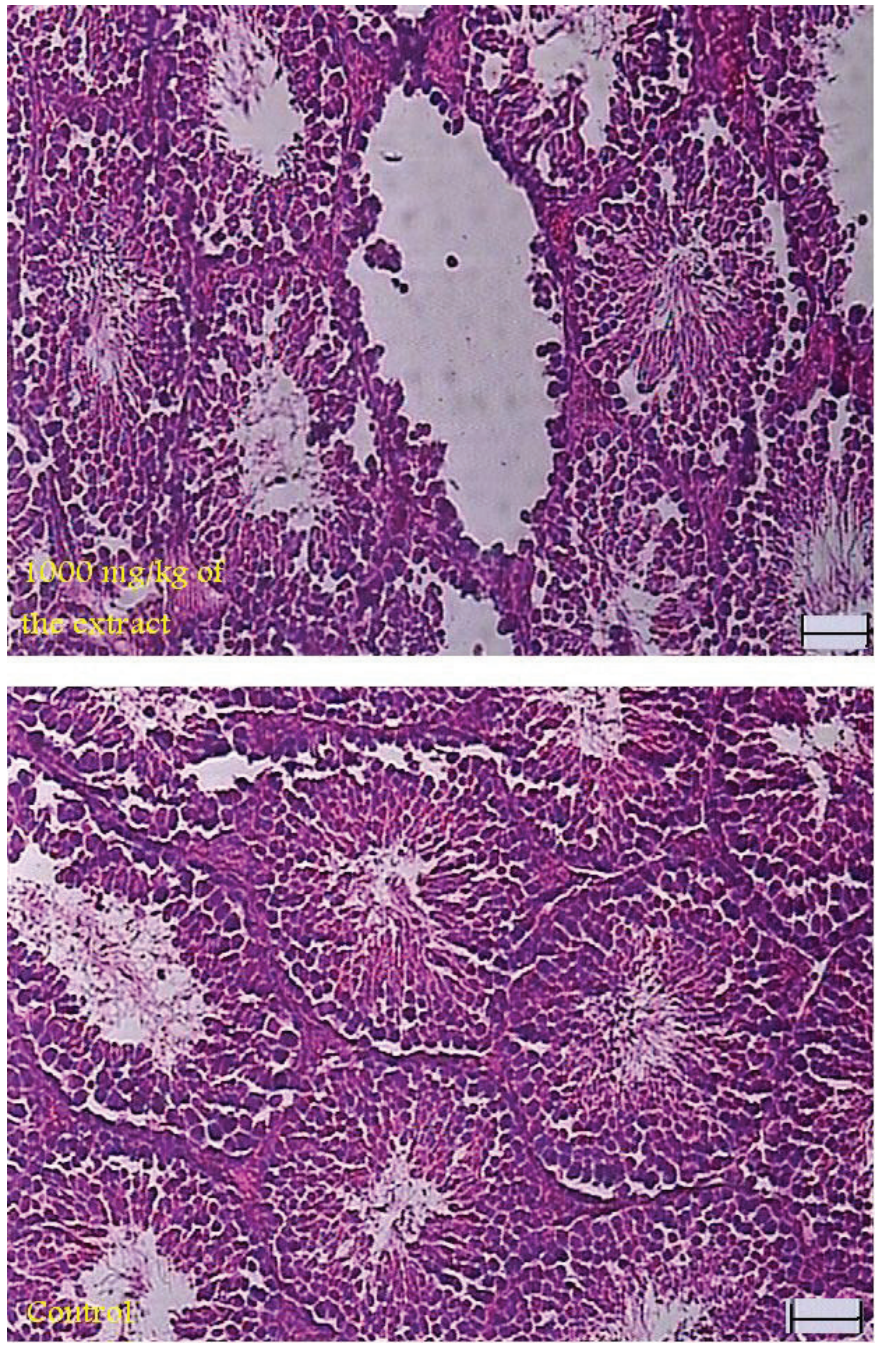

Figure 3. The micrograph of the testes removed from animals treated with 1000 mg/kg of extract and vehicle (negative control). Some seminiferous tubules of extract-treated animals showed degenerative changes compared to control testis. H & E stain. Scale bar=50µm.

Aphridite-treated animal seminiferous tubules showed a higher sperm density. No significant changes in Leydig and Sertoli cell density were detected in aphridite-fed groups (positive control) when compared to experimental and negative control group. Similar histology was observed in Seminal vesicle, epididymis and prostate of extract or aphrodite administrated group.

Discussion

Ethanolic extract of *S. aromaticum* can enhance male sexual activity. Therefore, it has been used in herbal medicine as an aphrodisiac in such cases of male sexual disorders or debility (Tajuddin et al. 2003). However, Mishra et al (2008) showed the *S. aromaticum* extract impacted the spermatogenesis and sex hormone levels in a dose dependent manner. It was shown that the serum testosterone concentration and spermatogenesis increase in mice treated with low dose of the *S. aromaticum* but they decline in high dose (Mishra and Singh 2008). Although, *S. aromaticum* extract and clove oil contain antioxidants that cure sperms quality theorically (Gulcin 2011), both of them are spermicidal (Buch et al. 1998).

In the present study, *S. aromaticum* extract reduced serum testosterone concentration and sperm density in seminiferous tubules in high dose-treated mice. Our data differs from the results obtained by Yakubu et al (2011) who used the same doses of the extract on rats for a shorter period of time and at doses of 500 and 1000mg/kg, which increased serum testosterone concentration (Yakubu and Akanji 2011). However, the data of this study confirms the finding obtained by Mishra and Singh (2008) who used the same duration as we fed the mice (Mishra and Singh 2008) . High dose extract administration also caused a significant increase in the serum level of estradiol. Reduction in testosterone and increase in estradiol levels may lead to a decrease in sperm count and motility.

Syzygium aromaticum extract administration has been shown to lead to a significant reduction in the number of proliferating cells and an increase in the number of apoptotic cells in the mice with induced lung cancer (Banerjee et al. 2006). Anti-proliferative activity of *S. aromaticum* extract may cause a decline in spermatogenesis and consequent to this, lead to a reduction in sperm count. A decline in daily sperm production was shown by *S. aromaticum* extract administration (Mishra and Singh 2008). Although, the mechanism is not clear, it seems that the *S. aromaticum* components interfere with spermatogenesis.

Eugenol, the major component of the *S. aromaticum*, caused the epithelial cells of the seminal vesicle to degenerate and to desquamate and consequently its secretary activity was diminished (Vanitakumari et al. 1998). However, in our study no histological changes were observed in seminal vesicle of extract-fed mice.

Aphredit (positive control) led to an increase in sperm count and motility compared with the negative control groups and extract-treated groups. The drug contains the extract of four herbs; *Troiblus terrestris, Zingiber officinate, Cricus sativus and Cinnamomum zeylanicum* (Sweetman 2009). *Syzygium aromaticum* extract also act like aphridite as Aphrodisiac agent (Tajuddin et al. 2003).

Our data showed a significant decrease in sperm parameters and the serum testosterone level extract administration. The extract may influence blood flow or nervous tissue and in this way it may improve reproductive activity but diminished spermatogenesis was observed in our data.

In conclusion, the data of the present study indicates that the high dosage of the *S. aromaticum* extract has a toxic effect on spermatogenesis. It reduces sperm count, motility and sperm density in the seminiferous tubules and it may be attributed to the effects of the extract on sex hormones concentration.

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