Reproductive effects of lipid soluble components of *Syzygium aromaticum* flower bud in male mice

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ABSTRACT

Background: The flower buds of *Syzygium aromaticum* (clove) have been used in indigenous medicines for the treatment of male sexual disorders in Indian subcontinent. **Objective:** To evaluate the effect of *Syzygium aromaticum* flower bud on male reproduction, using Parkes (P) strain mice as animal model. **Materials and Methods:** Mice were orally administered lipid soluble components of *Syzygium aromaticum* flower bud in doses of 15, 30, and 60 mg/kg body weight for 35 days, and several male reproductive endpoints were evaluated. **Results:** Treatment with lower dose (15 mg) of *Syzygium* increased the motility of sperm and stimulated the secretory activities of epididymis and seminal vesicle, while higher doses (30 and 60 mg) had adverse effects on sperm dynamics of cauda epididymidis and on the secretory activities of epididymis and seminal vesicle. Libido was not affected in treated males; however, a significant decrease in litter in females sired by males treated with higher doses of *Syzygium* was recorded. **Conclusion:** Treatment with *Syzygium aromaticum* flower bud causes dose-dependent biphasic effect on male reproductive indices in P mice; lower dose of *Syzygium* appears stimulatory, while the higher doses have adverse effect on male reproduction. The results suggest that the lower dose of *Syzygium* may have androgenic effect, but further studies are needed to support this contention.

Key words: Biphasic effect, epididymis, fertility, seminal vesicle, *Syzygium aromaticum*

INTRODUCTION

In modern society, incidence of male infertility has increased because of several factors including environmental pollution, stress, and lifestyle. Male infertility has both social and psychological impact on normal life, because an infertile man is considered as an incomplete man in Indian subcontinent.[1] Despite impressive technical advances in male reproductive health, treatment for male infertility in developing countries is beyond the reach of a common man as it is fairly expensive. The use of natural products with therapeutic properties is as ancient as human civilization, and even at present 70-80% of the world population continues to rely on nonconventional medicines of herbal origin for their primary healthcare needs.[2]

The flower bud of *Syzygium aromaticum* (L.) Merr. and Perry. (Family Myrtaceae), commonly known as clove, is a well-known traditional medicine in Australia, Southeast Asia and in Indian subcontinent, and this is widely used in various disorders including dental, respiratory, headache and soar throat.[3-5] In Ayurvedic and Unani medicines, the *Syzygium aromaticum* is well-known for its aphrodisiac property and is used to treat male sexual disorders.[5-7] Treatment with *Syzygium aromaticum* has been reported to produce a sustained increase in the mounting frequency of normal male rats and mice.[6,8] However, to the best of our knowledge, the effect of *Syzygium aromaticum* on functional physiology of the male reproductive organs has not been studied.

Considering the medicinal importance of flower bud oil and its constituents, we have used hexane to extract lipid soluble ingredients of flower bud of *Syzygium aromaticum* (SAx). We have performed microscopic, biochemical, and fertility studies after chronic oral exposure of lipid soluble ingredients of flower bud of *Syzygium aromaticum* (SAx) to investigate its effect on functional physiology of epididymis

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Received: 10-Nov-2012
Revised: 26-Dec-2012
Accepted: 11-Mar-2013

Access this article online

Quick Response Code:

Website: www.jaim.in

DOI: 10.4103/0975-9476.113870

and seminal vesicle, and on male fertility, using Parkes strain mice as animal model.

MATERIALS AND METHODS

Preparation of plant material

The sun-dried unopened flower buds of *Syzygium aromaticum*, after due authentication, were collected from Kerala (South India). The flower buds were coarsely ground; for extraction of lipid soluble ingredients, the ground plant material (250 g) was extracted with hexane in a soxhlet extractor for 24 h (12 × 2). The solvent was evaporated in solvent recovery apparatus to collect the extract (~12% of ground plant material) in form of dark brown viscous oil (SAx). This was stored at 4°C in an air tight container and dissolved in olive oil for experimentation.

Animals and treatment

Thirty-two (age: 14-15 weeks, weight 32-34 g) male laboratory mice of Parkes (P) strain from closed and randomly bred colony[9] were used in this study. Mice were randomly allocated into four groups (eight animals per group), with mice of same body weights in a group. Each group of experimental animals was housed separately in polypropylene cages (430 × 270 × 150 mm) with rice husk as bedding material. Body weight and general health of the animals were monitored regularly throughout the experimental period. Animals were maintained according to the guidelines of Institutional Animal Ethics Committee. The animals were fed olive oil or doses of SAx through gavage for 35 days (duration of one spermatogenic cycle in mouse) as follows:

Group I: Vehicle controls (olive oil, 200 μL/mouse/day
Group II: *Syzygium aromaticum* lipid component (15 mg/kg body weight/day)
Group III: *Syzygium aromaticum* lipid component (30 mg/kg body weight/day) and
Group IV: *Syzygium aromaticum* lipid component (60 mg/kg body weight/day).

The doses of *Syzygium* were selected based on a pilot study conducted in our laboratory in *P* mice. The volume of SAx along with olive oil fed to treated mice (Groups II-IV) was same to that of controls (Group I).

Autopsy

All animals were sacrificed 24 hours after the last treatment by decapitation. At autopsy, the body weight of all animals was recorded. The epididymis and seminal vesicle were excised, blotted free of blood and weighed to nearest 0.10 mg. Six epididymes from left or right sides of six mice were immediately used for sperm analyses, while the contralateral epididymis was used for sialic acid estimation. Seminal vesicle was used for fructose estimation.

Biochemical estimations

The concentration of sialic acid in the epididymis was estimated according to Aminoff's[10] thiobarbituric acid method and that of fructose in the seminal vesicle was determined by the method of Lindner and Mann.[11]

Sperm analyses

At autopsy, spermatozoa were obtained from cauda epididymidis of each mouse in 500 μL Dulbecco's modified eagle medium maintained at 37°C. Motility and number were assessed according to WHO laboratory manual;[12] for evaluation of morphologically abnormal sperm, criteria of Wyrobek and Bruce[13] and Zaneveld and Polakoski[14] were employed.

Fertility studies

The fertility of treated males was assessed through natural mating. For this, four days prior to cessation of treatment, each male of all the groups was paired with a virgin, regularly cycling female. Mating was confirmed by the presence of vaginal plug. The day on which plug was observed was considered as day one of pregnancy. The mated females were allowed to complete the term for litter. Following indices were calculated as follows:

$$\text{Index of libio} = \frac{\text{Number of males mated}}{\text{Number of males paired}} \times 100$$

$$\text{Male fertility index} = \frac{\text{Number of females pregnent}}{\text{Number of males paired}} \times 100$$

Statistical analyses

All data, except for body weight and fertility test results, were analyzed by one-way analysis of variance (ANOVA) followed by Newman-Keuls' multiple range test. Data on male fertility were analyzed by the Chi-square test. Student's *t*-test was used to analyze data on body weight. Differences were considered significant at $P < 0.05$.

RESULTS

Body weight and reproductive organs weight

No significant differences were found between the initial and final body weights of SAx-treated animals and controls; all animals maintained a healthy appearance throughout the period of investigation. However, significant reductions were noted in the weights of epididymis and seminal vesicle in mice treated with higher doses (30 and 60 mg) of SAx compared to controls [Table 1].

Sperm analyses

There was a marginal increase in sperm parameters in cauda epididymidis of mice treated with 15 mg dose of SAx than in controls. On the other hand, however,

Table 1: Effect of *Syzygium aromaticum* flower bud extract on body weight, organs weight, sperm dynamics, concentrations of sialic acid in the epididymis and fructose in seminal vesicle, and on fertility of male mice

Groups	Body weight (g)		Organs weight (mg)		Sperm dynamics			Sialic acid (μ moles/ 100 g tissue)	Fructose (μg/100 mg tissue)	Fertility			Litter/ female
	Initial	Final	Epididymis	Seminal Vesicle	Motility	Abnormal morphology (%)	Number ($\times 10^6$)			Tested	Mated	Fertile	
I Olive oil	32.00± 0.00	32.00± 0.58	35.40± 2.81	130.81± 6.84	79.8± 2.33	18.8± 2.20	10.7± 0.81	63.77± 4.40	480.86± 28.85	8	8	8	5.9± 0.73
II 15 mg SAx	32.00± 0.00	32.60± 0.89	34.90± 2.39	135.72± 7.88	83.1± 2.21	19.6± 2.88	10.6± 1.84	68.72± 5.77	491.61± 63.35	8	8	8	6.1± 0.99
III 30 mg SAx	32.00± 0.00	33.00± 1.06	25.84*± 1.73	117.26*± 5.70	53.3*± 2.51	31.2*± 4.08	4.6*± 1.48	47.64*± 8.41	430.09*± 27.52	8	8	5#	2.6*± 1.10
IV 60 mg SAx	34.00± 0.00	33.40± 1.14	22.96*± 2.71	101.22*± 7.88	44.3*± 4.98	37.2*± 4.43	3.6*± 0.78	39.07*± 8.03	351.74*± 40.26	8	8	3#	2.00*± 1.00

Values are mean±SD for six animals, *Significantly different from controls (P<0.05) by ANOVA followed by Newman-Keuls' multiple range test, #Significantly different from controls (P <0.05) by Chi-square test

significant reductions were found in motility and number of spermatozoa in cauda epididymidis of mice treated with 30 and 60 mg doses of SAx compared to controls, while a significant increase was noted in the number of morphologically abnormal spermatozoa in these mice compared to controls [Table 1].

Biochemical analyses

Sialic acid concentration in epididymis and that of fructose in seminal vesicle in mice treated with 15 mg dose of SAx showed an increase, while a significant decrease was noted in the concentrations of these constituents in mice treated with 30 and 60 mg doses of SAx compared to controls [Table 1].

Fertility

Treatment had no effect on libido of males in any of the treated groups; however, fertility of treated males was significantly compromised in 30 and 60 mg doses groups of SAx-treated mice [Table 1 and Figure 1]. Further, litter in females inseminated by males treated with lower dose of SAx showed an increase; however, litter in females inseminated by males treated with 30 and 60 mg doses of SAx, were significantly suppressed in comparison to controls [Table 1 and Figure 1].

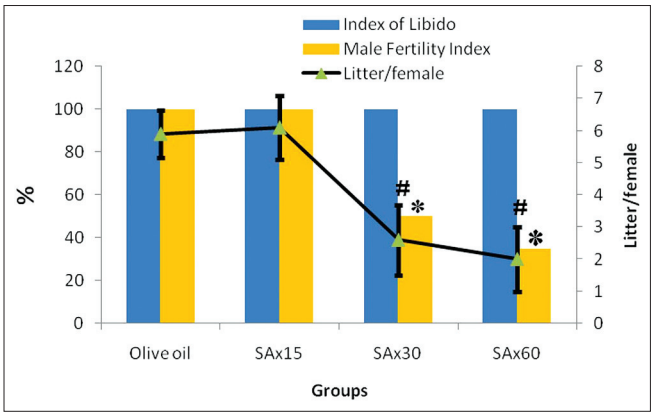

Figure 1: Effect of SAx treatment on index of libido, male fertility index and on number of litter from females sired by treated males. Values are mean ± S.D.; $n = 8$, * Significantly different from controls ($P < 0.05$) by Chi-square test, # Significantly different from controls ($P < 0.05$) by ANOVA followed by Newman-Keuls' multiple range test

DISCUSSION

Clove (flower bud of *Syzygium aromaticum*) oil is widely used for the treatment of various disorders, and the health benefits of clove oil are attributed to its antimicrobial, antifungal, antiseptic, antiviral, aphrodisiac, and stimulating properties. Keeping in view the medicinal importance of clove oil and its constituents, we have used hexane extract of *Syzygium aromaticum* flower bud to obtain lipid soluble components. In an earlier study with *Syzygium aromaticum,* we have reported our results in Parkes mice with respect to testicular function.[9] The present study is in continuation of the same and deals with the effect of *Syzygium* on functional physiology of epididymis and seminal vesicle, and on fertility of male mice.

The results of the present study indicate that *Syzygium aromaticum* treatment did not cause alterations in body weight of the treated animals, suggesting that the treatment had no systemic toxic effect in *P* mice. Mammalian epididymis plays a significant role in functional maturation of spermatozoa, and an optimal level of sialic acid (a true secretory product of epididymis) is essential for the functional integrity of spermatozoa.[15,16] A marginal increase in motility of spermatozoa in mice treated with lower dose of *Syzygium aromaticum* than in controls might be due to improvement in functional environment of the epididymis as there was also an increase in epididymal sialic acid concentration in the

treated mice. On the other hand however, higher doses of *Syzygium aromaticum* significantly decreased the motility and number of spermatozoa in the epididymis and also the sialic acid level in the duct; an adverse effect of higher doses of *Syzygium aromaticum* is also evident on sperm morphology as there was a significant increase in morphologically abnormal spermatozoa in treated mice compared to controls. A marked decrease in the number of spermatozoa in cauda epididymidis of mice treated with higher doses of *Syzygium aromaticum* is likely to be caused by the suppressive effect of SAx on spermatogenesis,[9] while alterations in sperm motility and morphology might have resulted from disturbances in functional environment of epididymis in these animals.[17] It is pertinent to mention here that Buch *et al.*,[18] have also reported spermicidal property of *Syzygium aromaticum* oil on ejaculated human spermatozoa.

SAx did not affect the libido of males in any of the treated groups. Further, there was a mild increase in weight of seminal vesicle and its fructose concentration, and in litter in females sired by males treated with lower dose of SAx, while at higher doses of the extract these parameters were significantly reduced in treated mice than in controls. Fructose is a good marker of seminal vesicle function.[19] Thus, poor fertility as observed in the present study at higher doses of SAx may be attributed to the adverse effects of SAx on spermatogenesis and on epididymis and seminal vesicle.

Secretory activities of epididymis and seminal vesicle are androgen-dependent.[20] SAx stimulated testosterone biosynthesis in lower dose, while suppressed the same at higher doses.[9] The increase in sperm motility and the secretory activities of epididymis and seminal vesicle (though not statistically significant) might be due to the androgenic nature of the extract, especially in lower dose; the higher doses of SAx, however, caused adverse effects on epididymal and vesicular functions. Thus, dual effects suggest the biphasic nature of the SAx at different dose levels. However, it is not clear whether the effect of *Syzygium aromaticum* on epididymis and seminal vesicle of mice is caused by eugenol or by other bioactive molecules of the extract. It is pertinent to mention here that treatment with eugenol is reported to cause decrease in the secretory activity of seminal vesicle as evident from decreased fructose level in the gland.[21] In the present study, higher doses of SAx adversely affected fructose concentration in seminal vesicle of treated mice; thus, the possibility that the eugenol might have a role in causing alterations in the reproductive organs of *P* mice cannot be altogether ruled out.

CONCLUSION

The results in *P* mice suggest biphasic nature of SAx on the male reproductive indices. Treatment with lower dose (15 mg) of SAx appears stimulatory in nature, while in higher doses (30 and 60 mg) it causes adverse effect on the reproductive indices. Further studies are, however, needed for a better understanding of the effect of *Syzygium* on the functional physiology of the male reproductive system.

ACKNOWLEDGMENTS

This work was supported by the Department of Science and Technology (DST), New Delhi, in the form of SERC FAST Track Project (No. SR/FT/L-72/2005) and CAS in Zoology, Banaras Hindu University.

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How to cite this article: Mishra RK, Singh SK. Reproductive effects of lipid soluble components of *Syzygium aromaticum* flower bud in male mice. J Ayurveda Integr Med 2013;4:94-8.

Source of Support: This work was supported by the Department of Science and Technology (DST), New Delhi, in the form of SERC FAST Track Project (No. SR/FT/L-72/2005) and CAS in Zoology, Banaras Hindu University, **Conflict of Interest:** None declared.