ANDROLOGY

Inhibition of Human Sperm Motility by Specific Herbs Used in Alternative Medicine

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Purpose: Our purpose was to analyze sperm motility parameters in the presence of herbs.

Methods: Washed sperm were incubated in either saw-palmetto (*Serenoa repens*, Permixon Sabal serrulatum), *echinacea purpurea*, *ginkgo biloba*, *St. John's wort* (*Hypericum perforatum*), or control medium. Parameters were measured on a Hamilton-Thorn analyzer after 1, 4, 24, and 48 hr at 37°C.

Results: Sperm motility was inhibited at the high concentration (0.6 mg/mL) of *St. John's wort*. Curvilinear velocities and beat cross frequencies also decreased, but not hyperactivation. High-concentration saw-palmetto, *echinacea*, or *ginkgo* inhibited motility at 24 and 48 hr.

Conclusions: A potent inhibition of sperm motility was seen in *St. John's wort* unrelated to changes in pH. Furthermore, sperm viability was compromised in *St. John's wort*, suggesting a spermicidal effect. Metabolic changes were observed in saw-palmetto-treated sperm. High-concentration *echinacea purpurea* interfered with sperm enzymes. *Ginkgo* did not have an antioxidant effect on sperm motility.

KEY WORDS: sperm; *ginkgo biloba*; *echinacea purpurea*; saw-palmetto; *St. John's wort*; herbs; alternative medicine.

INTRODUCTION

There has been considerable interest in the use of herbs and their extracts for the treatment of medical ailments.

St. John's wort (*Hypericum perforatum*), a herbal plant that grows in the western United States, Europe, North Africa and Asia is believed to have antidepressant activity acting on the central nervous system similar to selective serotonin reuptake inhibitors (SSRIs) (1,2). Saw-palmetto (*Serenoa repens*, *Sabal serrulatum*) berries from the palm tree native to Florida and the liposterolic extract (Permixon, Sereprostat, LESSr) are used to treat prostatic hyperplasia due to the antiandrogenic activity (3–5).

Echinacea purpurea is derived from the root of the purple coneflower and is used to enhance immunocompetent cell, peripheral blood mononuclear cell, or natural killer (NK) cell activity against bacteria and viruses such as herpes and influenza (6). The antitumor properties of *echinacea* have also been investigated (7). *Ginkgo biloba* extract from the oldest tree species in Asia is marketed as an antioxidant as well as for the treatment of memory loss, Alzheimer, vertigo, tinnitus, and asthma (8,9). However, studies focused on the effects of these herbs on the sperm cells are lacking. In the present study, the hypothesis is that the herbs do not affect sperm. The objective was to analyze the motility parameters of sperm cells incubated in the presence of different types and concentrations of herbs. The results obtained will help to determine the impact of these herbs on male reproductive cells.

MATERIALS AND METHODS

The design of the study involved measuring kinematic parameters of sperm exposed to different types and concentrations of popular herbs used in alternative medicine. Fresh donor sperm cells were used. The

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sperm kinematic parameters were measured with a Hamilton Thorn computer-aided sperm analyzer (CASA).

Preparation of Sperm

Fresh sperm specimens were washed using the two-layer colloid (Isolate; Irvine Scientific, Santa Ana, CA) solution procedure (10). The experiments were done in replicates and a single fertile donor was used to limit inter donor variability in sperm. The colloid wash procedure consisted of layering the semen on top of a discontinuous two-layer colloid gradient in a 15-ml centrifuge tube. The bottom layer consisted of 1.5 ml of the lower, more concentrated colloid solution, while the next layer was the 1.5 ml of upper colloid layer. The gradients were centrifuged at 300g for 20 min and the bottom layer (1.5 ml) along with the pellet were added with an equal volume of (*N*-(2-hydroxy ethyl) piperazine-*N'*-2-ethanesulfonic acid (HEPES)-buffered synthetic human tubal fluid (modified HTF) medium. The mixtures were recentrifuged and the final pellets were resuspended at a concentration of 10 million sperm/ml and divided into several tubes.

Stock herbal solutions consisted of each of the four herbs dissolved in modified HTF and filtered through 0.8- μ m filters. These were added separately to each sperm suspension. Plain modified HTF served as the control. Protein supplementation was absent from the preparations to reduce confounding factors. However, 10% human serum albumin in modified HTF was used to wet the glass slides prior to microscopic analyses at room temperature to prevent sperm sticking to the glass slides. The sperm were incubated at 37°C for 48 hr. Analyses based on aliquots of the sperm were carried out after 0, 1, 4, 24, and 48 hr of incubation.

The saw-palmetto (*Permixon*, *Serenoa repens*) was tested at 0.9 and 9.0 mg/ml. The ginkgo biloba was tested at 0.12 and 1.2 mg/ml, while the echinacea purpurea was tested at 0.81 and 8.1 mg/ml. Finally, St. John's wort (*Hypericum perforatum*) was tested at 0.06 and 0.6 mg/mL. With the exception of St. John's wort, the low concentrations were based on one-thousandth of the recommended daily dose dissolved in 1 ml of medium. The low concentration of St. John's wort was based on one-millionth of the recommended daily dose dissolved in 1 mL of medium.

Computer-Aided Sperm Analyses

Sperm dimensions and kinematic parameters were measured using the Hamilton Thorn motility analyzer

(HTM-C) CASA system as described previously (10). Briefly, the analyses were carried out using aliquots (10 μ l each) of the treated sperm specimens in 20- μ m-deep glass slides with cover slips prewarmed at 37°C on a slide warmer. A minimum of 100 sperm cells was analyzed each time. Analyses were carried out after 0, 1, 4, 24, and 48 hr of incubation. Sperm hyperactivation motility was defined as whiplash movement with a wide amplitude of lateral head displacement, high curvilinear velocity, and low or non-linear direction (11). The percentage of hyperactive sperm was measured using the sort fraction module of the HTM-C based on published settings on this analyzer (10).

Statistical Analyses

The results (Tables I and II) are presented as the mean $\pm$ 1 standard deviation. Means derived from sperm sampled over a period were assessed using the repeated-measures analysis of variance (ANOVA) where the fixed effects were concentration of herbs (low or high dosages) and exposure time (1, 4, 24, and 48 hr), followed by significance testing using Tukey's *Q* test. Student's *t*-test was used whenever appropriate. A *P* value or less than 0.05 value was considered significant.

RESULTS

Sperm motility was inhibited in vitro in the presence of the low concentration (0.06 mg/ml) of St. John's wort from 24 hr onward (Table I). In contrast, the high concentration (0.6 mg/ml) of St. John's wort had an immediate inhibitory effect on sperm motility from 1 hr onward (Table I). The decrease seen in the total sperm motility was also mirrored in decreases in the curvilinear velocities and beat cross frequencies. Interestingly, there were no differences in hyperactivation and in the other kinematic parameters.

There were no significant differences in the motility and the kinematic parameters among the remaining three herbs, saw-palmetto, echinacea, and ginkgo, versus the control at the low concentration tested (Table I). However, when the concentration was increased, sperm motility was inhibited after 48 hr in saw-palmetto (Table II). Sperm motility was inhibited after 24 hr in the presence of the high concentrations of echinacea and ginkgo. Sperm curvilinear velocities were lower after 24 hr in the high concentration of echinacea, ginkgo, and St. John's wort compared with

Table I. Analysis of Human Sperm Parameters in the Presence of Different Types of Herbs^a

	Control (n = 205)	Saw-palmetto (0.90 mg/ml) (n = 204)	Echinacea (0.81 mg/ml) (n = 207)	Ginkgo (0.12 mg/ml) (n = 203)	St. John's wort (0.06 mg/ml) (n = 203)
Percentage total motility (%)					
Hour 1	82.5 ± 2.5	84.0 ± 2.0	84.0 ± 3.0	86.0 ± 3.0	76.0 ± 13.0
Hour 4	76.5 ± 1.5	73.0 ± 6.0	88.5 ± 3.5	79.5 ± 1.5	69.0 ± 8.0
Hour 24	67.5 ± 9.5	67.0 ± 4.0	59.5 ± 4.5	56.0 ± 5.0	24.5 ± 14.5*
Hour 48	61.5 ± 2.5	52.0 ± 7.0	5.5 ± 1.5*	10.5 ± 1.5*	0 ± 0*
Curvilinear velocity (μm/sec)					
Hour 1	55.0 ± 5.0	45.5 ± 3.5	53.5 ± 6.5	54.5 ± 4.5	52.5 ± 4.5
Hour 4	47.5 ± 1.5	43.0 ± 3.0	66.0 ± 4.0*	56.0 ± 5.0	47.5 ± 3.5
Hour 24	36.5 ± 2.5	40.0 ± 2.0	42.5 ± 0.5	40.5 ± 0.5	27.5 ± 3.5*
Hour 48	39.0 ± 0	42.0 ± 7.0	30.5 ± 2.5	35.0 ± 6.0	0 ± 0*
Hyperactivation (%)					
Hour 1	1.5 ± 1.5	0.5 ± 0.5	1.0 ± 1.0	0.5 ± 0.5	2.0 ± 2.0
Hour 4	0.5 ± 0.5	0.5 ± 0.5	2.0 ± 0	1.0 ± 0	0.5 ± 0.5
Hour 24	0 ± 0	0.5 ± 0.5	1.0 ± 1.0	0 ± 0	0 ± 0
Hour 48	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Beat cross frequency (Hz)					
Hour 1	8.9 ± 0	6.7 ± 1.1*	7.4 ± 1.1	7.3 ± 0.5	8.8 ± 0.4
Hour 4	8.3 ± 0.1	8.0 ± 1.1	11.1 ± 0.8*	10.9 ± 1.5*	8.4 ± 2.0*
Hour 24	7.2 ± 0.3	8.5 ± 0.1	8.5 ± 0.3	8.3 ± 1.2	9.0 ± 0.2*
Hour 48	8.0 ± 0.8	8.4 ± 0.3	7.4 ± 4.9	10.0 ± 3.7*	0 ± 0*

^a Mean ± 1 standard deviation.* $P < 0.05$.

the control. The beat cross frequencies were higher for the high concentration of saw-palmetto, echinacea, and St. John's wort treatment groups after 4 hr of incubation. There were no differences in the other remaining kinematic parameters for the high concentrations of saw-palmetto, echinacea, and ginkgo.

DISCUSSION

The present in vitro study demonstrated a potent inhibitory effect on sperm motility in the presence of St. John's wort (*Hypericum perforatum*). Even at the lower concentration, an inhibitory effect was observed from 24 hr onward. The sperm curvilinear velocity, which has been correlated with the sperm fertilizing potential, was also decreased by St. John's wort treatment. This herb plant, which grows in the western United States, Europe, North Africa, and Asia, is prescribed for the alleviation of depression (1,2) and is said to impart a feeling of well-being to the treated person. The mechanism involved in the reduction of sperm motility by this herb remained unknown but it did not involve changes in pH. However, a check of

sperm viability after 48 hr in St. John's Wort showed only 16% viable sperm, compared with 55% viable for the control sperm, suggesting a spermicidal effect of St. John's Wort. To the best of our knowledge, no data exist on the concentrations of the herbs in semen or serum. The concentrations chosen in this study were based on fractions of the recommended daily doses. The possibility of a lack of a physiological effect in vivo should be considered. Furthermore, the active components of the herbs responsible for the observed effects were not determined, as they were beyond the scope of this study and will be scrutinized in future experiments.

The saw-palmetto popularly used to treat benign prostatic hyperplasia has been shown to have antiandrogenic activity (3–5,12). Sperm motility parameters were not significantly affected by saw-palmetto. Since saw-palmetto acts by inhibiting the production of 5-lipoxygenase metabolites (4), it stands to reason why saw-palmetto did not have a pronounced effect on the ejaculated sperm cell, which lacks the cytoplasmic components involved in the arachidonic pathways. Note that at the high dose and after 48 hr, there is a decrease in motility without a change in curvilinear

Table II. Analysis of Human Sperm Motility in the Presence of Different Types of Herbs^a

	Control (n = 205)	Saw-palmetto (9.0 mg/ml) (n = 203)	Echinacea (8.1 mg/ml) (n = 210)	Ginkgo (1.2 mg/ml) (n = 219)	St. John's wort (0.6 mg/ml) (n = 202)
Total motility (%)					
Hour 1	81.0 ± 1.0	91.5 ± 1.5	82.0 ± 4.0	88.5 ± 1.5	36.0 ± 13.0*
Hour 4	77.5 ± 7.5	68.5 ± 1.5	84.5 ± 0.5	70.5 ± 10.5	16.5 ± 2.5
Hour 24	64.5 ± 2.5	64.5 ± 11.5	15.5 ± 4.5*	0 ± 0*	0 ± 0*
Hour 48	57.5 ± 2.5	10.0 ± 9.0*	0 ± 0*	0 ± 0*	0 ± 0*
Curvilinear velocity (µm/sec)					
Hour 1	45.5 ± 3.5	59.0 ± 1.0*	55.0 ± 0*	47.0 ± 7.0	38.0 ± 8.0*
Hour 4	40.0 ± 4.0	50.5 ± 1.5	55.5 ± 3.5*	41.0 ± 6.0	30.5 ± 6.5*
Hour 24	37.5 ± 3.5	47.5 ± 8.5	28.5 ± 6.5*	0 ± 0*	0 ± 0*
Hour 48	35.5 ± 1.5	30.0 ± 5.0	0 ± 0*	0 ± 0*	0 ± 0*
Hyperactivation (%)					
Hour 1	1.0 ± 1.0	2.0 ± 1.0	1.0 ± 1.0	0.5 ± 0.5	1.0 ± 1.0
Hour 4	0.5 ± 0.5	2.5 ± 1.5	1.0 ± 1.0	1.0 ± 1.0	0 ± 0
Hour 24	0.5 ± 0.5	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Hour 48	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Beat cross frequency (Hz)					
Hour 1	8.6 ± 2.5	11.3 ± 1.1*	10.0 ± 2.4*	9.6 ± 3.8	6.2 ± 3.0*
Hour 4	7.0 ± 0.9	8.5 ± 2.1*	8.0 ± 3.2*	7.4 ± 2.7	8.5 ± 1.0
Hour 24	7.3 ± 0.8	10.1 ± 2.1*	9.5 ± 1.7*	0 ± 0*	0 ± 0*
Hour 48	6.8 ± 0	19.9 ± 10.1*	0 ± 0*	0 ± 0*	0 ± 0*

^a Mean ± 1 standard deviation.* *P* < 0.05.

velocity. The explanation for this remains unknown. A more important observation was that the beat cross frequency was increased at the high concentration of saw-palmetto, suggesting an effect on sperm metabolism unrelated to motility.

Echinacea purpura, which is claimed to be antibacterial, antiviral, and antitumor (6,7), did not have an effect on sperm motility parameters. Only at the high concentration and after 24 hr is an inhibitory effect seen with regard to sperm motility and velocities. This herb works by increasing the production of interferon and interleukins by immunologically competent cells. Another mechanism of action of echinacea is to inhibit hyaluronidase. However, one of the enzymes localized at the sperm head is hyaluronidase, which helps the sperm to penetrate the cumulus cell layers around the oocyte. Potentially, the presence of echinacea may prevent sperm from fertilizing the oocytes. More studies are in progress to examine this aspect of echinacea.

Ginkgo biloba, a tree extract from Asia, is used in the treatment of memory loss, Alzheimer, vertigo, tinnitus, and asthma (8,9). It has been claimed that ginkgo is also an antioxidant. Because oxidation of the sperm by free radicals results in damaged sperm membranes and damaged DNA, in theory, ginkgo

should protect the sperm. The data showed that ginkgo did not have an effect on sperm motility parameters except at the high concentration from 24 hr onward. Because the presence of ginkgo did not improve sperm parameters, there were no advantages of this herb for the sperm.

In summary, of the four herbs tested, St. John's wort seems to be the most potent in terms of inhibiting sperm motility. Since the sperm requires motility for transport, penetration of cumulus cell layers, and interaction with the oocyte, some aspects of fertility may be compromised, particularly in the presence of a high concentration of St. John's wort. Further studies are needed to determine the precise mechanism of action of this herb on the sperm cell.

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