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Effect of *Mucuna pruriens (Wanduru me)* on viability of sperm *in vitro*

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ABSTRACT

Introduction: Sub-fertility is defined as the failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse. Some herbal plants are used in Ayurvedic Medicine to treat sub-fertility. This study aimed to assess the effect of *Mucuna pruriens* on percentage of sperm viability reduction with time.

Methods: A total of 32 fertile males aged between 18-50 years were recruited for the study. Semen samples were collected. Samples were allowed to liquefy and then aliqoted. *Mucuna pruriens* water extractions and ethanol extractions were prepared. Aliqoted semen samples were incubated with 2 mg/mL, 1.5 mg/mL and 1 mg/mL concentration of *Mucuna pruriens* extractions for 90 minutes in 37˚C. The control was prepared by adding balanced salt solution to the sperm without adding *Mucuna pruriens*. Sperm viability in each solution was measured after 15 minutes, 45 minutes and 90 minutes. Same procedure was followed for 10 semen samples from sub-fertile men.

Results: There is a statistically significant difference in mean values of the sperm viability between the control samples and semen incubated with 2 mg/mL, 1.5 mg/mL and 1 mg/mL concentration of *Mucuna pruriens* water extractions. There is a statistically significant improvement of sperm viability with all concentrations of *Mucuna pruriens*. The highest percentage of sperm viability was demonstrated in 1.5 mg/mL of *Mucuna pruriens* distilled water extraction while there was no significant effect demonstrated with ethanol extractions.

Conclusions: Distilled water extraction of *Mucuna pruriens* seeds is effective in sustaining sperm viability until 90 minutes of incubation at 37°C.

Keywords: *Ethanol extraction, Mucuna pruriens, Sperm viability, Sub-fertility, Water extraction.*

Introduction

Subfertility is defined as the failure to achieve a clinical pregnancy after 12 months or more of frequent unprotected sexual intercourse. Subfertility has become a global health issue affecting individuals in reproductive age. World Health Organization (WHO) has estimated that one in every 6 individuals are suffering from subfertility (1). Evidence from the literature supports the fact that globally 60-80 million couples suffer from subfertility every year. The male subfertility is responsible for more than 20% of subfertility cases (1, 2). Similarly, a study conducted in Sri Lanka,

concluded that 26.4% cases of subfertility were caused by male factors, whereas 22.3% caused by female factors and 28.9% sub fertility due to combination of both male and female factors (3). The common reasons of male subfertility includes alteration in concentration, viability, motility and morphology of sperm (4).

Assisted reproductive techniques (ART) are widely used to treat couples with sub-fertility. In ART, semen needs to be processed *in vitro* and this procedure takes time. Therefore, the viability of sperms can be reduced during the ART and ultimately affect the success rate of these treatments (5). *Mucuna pruriens* (*Wanduru me*) has been used in Ayurveda Medicine to treat subfertility (1). A clinical trial reported that powder of *M. pruriens* seed significantly inhibit lipid peroxidation, elevate spermatogenesis and improve sperm motility (7). Similarly, another clinical trial concluded that treatment with powder of *M. pruriens* seed improved the semen quality and was capable of regulating steroidogenesis (8). Another study has revealed that *M. pruriens* seed powder reduced psychological stress and seminal plasma lipid peroxide levels in subfertile men. The same study further reported that seed powder improved sperm count and motility in subfertile men (9).

However, the studies conducted on *in vitro* effect of *M. pruriens* seed powder on sperm viability are sparse. There is no reported study on its effect on sperm viability in Sri Lanka. Therefore, the current study was planned to find the effect of *M. pruriens* seed powder on sperm viability *in vitro*.

Methods

A cross-sectional study with a control group was conducted to assess the effect of *M. pruriens* seed powder on percentage of sperm viability in a selected group of adult males. A total of 32 semen samples were collected from 32 fertile men and 10 additional semen samples were collected from 10 men diagnosed to have sub-fertility. *M. pruriens* plant with seeds was authenticated by the taxonomists of the National Herbarium, Peradeniya, Sri Lanka. Initially, a pilot study was conducted using 5 different concentrations of *M. pruriens* seeds distilled water and ethanol extractions (2 mg/mL, 1.5 mg/mL, 1 mg/L, 0.5 mg/dL and 0.25 mg/mL). Among them, 3 optimum *M. pruriens* seeds distilled water (2 mg/mL, 1.5 mg/mL and 1 mg/mL) and ethanol extractions (1 mg/mL, 0.5 mg/dL and 0.25 mg/mL) were selected for the study.

The collected semen samples were allowed to liquefy. Once liquefied, semen samples were separated into 200 µl aliquots. Then, 200 µl of semen was mixed with 200 µl of *M. pruriens* water extractions (2.0 mg/dL, 1.5 mg/dL and 1.0 mg/dL) and ethanol extractions (1.0 mg/dL, 0.5 mg/dL and 0.25 mg/dL) separately. These mixtures were incubated at 37°C for 90 minutes. However, semen samples collected from 10 men with sub-fertility were checked only with *M. pruriens* seeds distilled water (2 mg/mL, 1.5 mg/mL and 1 mg/mL). Control samples were prepared by mixing 200 µl of semen with 200 µl balanced salt solution. Controls were also incubated at 37°C for 90 minutes. The viability of sperms after incubation was assessed using Eosin-Nigrosin staining procedure. Each sample was examined by two examiners and the mean viability value was calculated. If the difference of two readings was less than 5%, average was taken as the final figure, and if the difference was more than 5% a third value was taken. The collected data were analysed using *SPSS* 25.0 statistical software. Ethical approval for the study was obtained from the Ethical Review Committee, Faculty of Allied Health Sciences, University of Ruhuna, Sri Lanka.

Results

Effect of *M. pruriens* water extraction on sperm viability in fertile men

After 15 minutes of incubation at 37°C, the mean ± SD viability percentage of sperm in control group was 52.06 ± 6.77. The mean viability percentage of the sperms incubated with 2.0 mg/dL, 1.5 mg/dL and 1.0 mg/dL of *M. pruriens* water extractions were 58.03 ± 6.31, 59.41 ± 7.45 and 47.78 ± 5.59 respectively.

After 45 minutes of incubation at 37°C, the mean viability percentage of sperm in control group was 47.78 ± 5.59. The mean viability percentage of the sperms incubated with 2.0 mg/dL, 1.5 mg/dL and 1.0 mg/dL of *M. pruriens* water extractions were 56.78 ± 7.15, 57.69 ± 8.70 and 55.16 ± 6.55 respectively.

After 90 minutes of incubation in 37°C, the mean viability percentage of sperm in control group was 45.25 ± 4.20. The mean viability percentage of the sperms incubated with 2.0 mg/dL, 1.5 mg/dL and 1.0 mg/dL of *M. pruriens* water extractions were 53.22 ± 7.30, 55.28 ± 6.88 and 53.09 ± 6.36 respectively.

After 15 minutes of incubation, there is a significant difference in mean viability percentage of the sperm incubated with 2.0 mg/dL, 1.5 mg/dL and 1.0 mg/dL *M. pruriens* water extractions compared to the control group (p<0.001).

Similarly, after 45 minutes of incubation, there is a significant difference in mean percentage viability of thesperm incubated with 2.0 mg/dL, 1.5 mg/dL and 1.0 mg/dL *M. pruriens* water extractions compared to control group (p<0.001). Interestingly, after 90 minutes of incubation, there is a significant difference in mean percentage viability of the sperm incubated with 2.0 mg/dL, 1.5 mg/dL and 1.0 mg/dL *M. pruriens* water extractions compared to control group (p<0.001). the findings are summarised in Table 1.

Table 1: Viability of sperm in different concentrations of *M. pruriens* water extractions: Comparison of samples obtained from fertile men and a control sample

Incubation time	Concentration of *Mucuna pruriens* water extractions	Mean ± SD viability percentage of sperm	Mean Difference of sperm viability compared to control group	Std. Error	*p* value	95% Confidence Interval	
						Lower Bound	Upper Bound
After 15 minutes incubation	2 mg/mL	49.40 ± 10.77	-10.50*	1.916	<0.001	-14.835	-6.165
	1.5 mg/mL	45.80 ± 10.72	-6.90*	2.627	0.027	-12.842	-0.958
	1 mg/mL	42.70 ± 11.07	-3.80	2.546	0.170	-9.560	1.960
	Control group	38.90 ± 8.50					
After 45 minutes of incubation	2 mg/mL	44.60 ± 9.36	-5.70*	1.904	0.015	-10.006	-1.394
	1.5 mg/mL	45.60 ± 9.32	-8.20*	2.107	0.004	-12.967	-3.433
	1 mg/mL	39.50 ± 15.28	-5.00*	2.165	0.046	-9.898	-0.102
	Control group	34.70 ± 9.51					
After 90 minutes of incubation	2 mg/mL	44.60 ± 9.36	-9.90*	2.818	0.007	-16.276	-3.524
	1.5 mg/mL	45.60 ± 9.32	-10.90*	2.392	0.001	-16.311	-5.489
	1 mg/mL	39.50 ± 15.28	-4.80	2.611	0.099	-10.707	1.107
	Control group	34.70 ± 9.51					

Effect of *Mucuna pruriens* water extractions on sperm viability in subfertile population

After 15 minutes of incubation at 37°C, the mean viability percentage of sperm in control group was 38.90 ± 8.50. The mean viability percentage of the sperms incubated with 2.0 mg/dL, 1.5 mg/dL and 1.0 mg/dL *M. pruriens* water extractions were 49.40 ± 10.77, 45.80 ± 10.72 and 42.70 ± 11.07 respectively.

After 45 minutes of incubation at 37°C, the mean viability percentage of sperm in control group was 40.90 ± 7.13. The mean viability percentage of the sperms incubated with 2.0 mg/dL, 1.5 mg/dL and 1.0 mg/dL *M. pruriens* water extractions were 46.60 ± 8.76, 49.10 ± 8.77 and 45.90 ± 10.04 respectively.

After 90 minutes of incubation at 37°C, the mean viability percentage of sperm in control group was 34.70 ± 9.51. The mean viability percentage of the sperms incubated with 2.0 mg/dL, 1.5 mg/dL and 1.0 mg/dL *M. pruriens* water extractions were 44.60 ± 9.36, 45.60 ± 9.32 and 39.50 ± 15.28 respectively.

After 15 minutes of incubation, there is a significant difference in mean percentage viability of the sperm kept in 2.0 mg/dL (p<0.001), 1.5 mg/dL (p=0.027) and 1.0 mg/dL (p=0.170) *M. pruriens* water extractions compared to control group. After 45 minutes of incubation, there is a significant difference in mean percentage viability of the sperm kept in 2.0 mg/dL (p=0.015), 1.5 mg/dL (p=0.004) and 1.0 mg/dL (p=0.046) *M. pruriens* water extractions compared to control group. After 90 minutes of incubation, there was a significant difference in mean percentage viability of the sperm kept in 2.0 mg/dL (p=0.007), 1.5 mg/dL (p=0.001) and 1.0 mg/dL (p=0.099) *M. pruriens* water extractions compared to control group. This indicates that in all given concentrations, *M. pruriens* has a significant impact on viability of sperms obtained from subfertile man compared to the control group. The findings are summarised in Table 2.

Table 2: Viability of sperm in different concentrations of *M. pruriens* water extractions: Comparison of samples obtained from subfertile men and a control sample

Incubation time	Concentration of *Mucuna pruriens* water extractions	Mean ± SD viability percentage of sperm	Mean Difference of sperm viability compared to control group	Std. Error	*p* value	95% Confidence Interval	
						Lower Bound	Upper Bound
After 15 minutes incubation	2 mg/mL	49.40 ± 10.77	-10.50*	1.916	<0.001	-14.835	-6.165
	1.5 mg/mL	45.80 ± 10.72	-6.90*	2.627	0.027	-12.842	-0.958
	1 mg/mL	42.70 ± 11.07	-3.80	2.546	0.170	-9.560	1.960
	Control group	38.90 ± 8.50					
After 45 minutes of incubation	2 mg/mL	44.60 ± 9.36	-5.70*	1.904	0.015	-10.006	-1.394
	1.5 mg/mL	45.60 ± 9.32	-8.20*	2.107	0.004	-12.967	-3.433
	1 mg/mL	39.50 ± 15.28	-5.00*	2.165	0.046	-9.898	-0.102
	Control group	34.70 ± 9.51					
After 90 minutes of incubation	2 mg/mL	44.60 ± 9.36	-9.90*	2.818	0.007	-16.276	-3.524
	1.5 mg/mL	45.60 ± 9.32	-10.90*	2.392	0.001	-16.311	-5.489
	1 mg/mL	39.50 ± 15.28	-4.80	2.611	0.099	-10.707	1.107
	Control group	34.70 ± 9.51					

Effect of *M. pruriens* ethanol extractions on sperm viability in the fertile population

After 15 minutes of incubation at 37°C, the mean viability percentage of sperm in control group was 56.84 ± 7.17. The mean viability percentages of the sperms incubated with 1.0 mg/dL, 0.5 mg/dL and 0.25 mg/dL *M. pruriens* ethanol extractions were 55.69 ± 7.48, 56.84 ± 6.82 and 55.87 ± 7.37 respectively.

After 45 minutes of incubation at 37°C, the mean viability percentage of sperm in control group was 51.97 ± 6.36. The mean viability percentage of the sperms incubated with 1.0 mg/dL, 0.5 mg/dL and 0.25 mg/dL *M. pruriens* ethanol extractions were 51.75 ± 6.33, 51.25 ± 6.47 and 51.41 ± 6.50 respectively.

After 90 minutes of incubation at 37°C, the mean viability percentage of sperm in control group was 47.53 ± 5.32. The mean viability percentage of the sperms incubated with 1.0 mg/dL, 0.5 mg/dL and 0.25 mg/dL *M. pruriens* ethanol extractions were 47.88 ± 5.29, 47.28 ± 5.01 and 46.78 ± 5.43 respectively.

After 15 minutes incubation, there was a significant difference in mean percentage viability of the sperm kept in 1.0 mg/dL (p=0.002), 0.5 mg/dL (p=0.004) and 0.25 mg/dL (p=0.004) *M. pruriens* ethanol extractions compared to control group. However, after 45 minutes incubation, there was no significant difference in mean viability percentage in sperm incubated with 1.0 mg/dL (p=0.304), 0.5 mg/dL (p=1.000) and 0.25 mg/dL (p=0.080) *M. pruriens* ethanol extractions compared to control group.

Similarly, after 90 minutes incubation, there was no significant difference in mean viability percentage in sperm incubated with 1.0 mg/dL (p=0.276), 0.5 mg/dL (p=0.429) and 0.25 mg/dL (p=0.112) *M. pruriens* ethanol extractions compared to the control group, Therefore, there was no improvement in viability in sperm incubated with *M. pruriens* ethanol extractions observed after 15 minutes.

Table 3: Viability of sperm in different concentrations of *M. pruriens* ethanol extractions: Comparison of samples obtained from fertile men and a control sample

Incubation time	Concentration of *Mucuna pruriens* ethanol extractions	Mean ± SD viability percentage of sperm	Mean Difference of sperm viability compared to control group	Std. Error	*p* value	95% Confidence Interval	
						Lower Bound	Upper Bound
After 15 minutes of incubation	1 mg/mL	55.69 ± 7.48	1.156*	0.348	0.002	0.446	1.866
	0.5 mg/mL	56.84 ± 6.82	0.719*	0.234	0.004	0.241	1.197
	0.25 mg/mL	55.87 ± 7.37	0.969*	0.313	0.004	0.331	1.606
	Control group	56.84 ± 7.17					
After 45 minutes of incubation	1 mg/mL	51.75 ± 6.33	0.219	0.209	0.304	-0.208	0.646
	0.5 mg/mL	51.25 ± 6.47	0.000	0.220	1.000	-0.449	0.449
	0.25 mg/mL	51.41 ± 6.50	0.563	0.311	0.080	-0.072	1.197
	Control group	51.97 ± 6.36					
After 90 minutes of incubation	1 mg/mL	47.88 ± 5.29	-0.344	0.310	0.276	-0.976	0.288
	0.5 mg/mL	47.28 ± 5.01	0.250	0.308	0.423	-0.378	0.878
	0.25 mg/mL	46.78 ± 5.43	0.750	0.458	0.112	-0.184	1.684
	Control group	47.53 ± 5.32					

Discussion

There has been a great deal of interest in using traditional medicines to replace synthetic drugs in the world. The WHO also encourages the use of medicinal plants, and/or compounds isolated from medicinal plants as a supplement or for the treatment of diseases (10).

Medicinal plants and phytotherapeutic compounds containing phenols are used to treat male subfertility (11). A randomised, placebo-controlled, double-blind clinical study conducted in Korea concluded that there is a significant improvement in sperm viability in subfertile patients treated with *Panax ginseng* (Korean Red Ginseng) (12). Another clinical trial reported that oral administration of 2 g capsule of *Nigella sativa* (black seed) for 3 months had significantly enhanced the sperm viability in subfertile men (13). A recent study reported that 0.2% of *Matricaria chamomilla* hydrolate could enhance the sperm viability (14).

The findings of the current study, show remarkable preservation of sperm viability in fertile men with all concentrations of *M. pruriens* distilled water extraction. The current study shows that the reduction of sperm viability is minimized by 1.5 mg/mL *M. pruriens* distilled water extraction. Although the viability of sperm samples was preserved in 2.0 mg/dL, 1.5 mg/dL and 1.0 mg/dL *Mucuna pruriens* seed powder distilled water extractions, 1.5 mg/mL is the best extraction for sperm viability preservation. Moreover, although *M. pruriens* seed powder distilled water extractions could preserve sperm viability up to 90 minutes, the sperm viability is best preserved up to 45 minutes. Hence, incubation of semen samples with *M. pruriens* seed powder distilled water extractions for 45 minutes is recommended. When considering the semen samples from subfertile men, water extractions of the *M. pruriens* demonstrated a similar pattern.

Several studies have tried to figure out effect of plant extract on sperm viability *in vitro*. One of them has reported that 40 µg/mL and 50 µg/ mL of *Tribulus terrestris* extract could enhance the sperm viability for 120 minutes (15). Another study conducted using *Cissampelos capensis* rhizome extract reported that Cissampelos capensis rhizome extract could not preserve sperm viability (16).

Several sperm preparation techniques are used in the ART such as classical swim-up method, density gradient centrifugation and filtration techniques. Classical swim-up method usually takes around 45 - 50 minutes. Similarly, both density gradient centrifugation and filtration techniques usually take 30 - 60 minutes (17). Since sperm preparation techniques takes time, sperm viability can be reduced at the end of the procedure and will ultimately affect the success rate of the ART. *M. pruriens* seed powder water extractions preserve sperm viability at its best up to 45 minutes and thereafter still capable of preserving viability upto 90 minutes. Therefore, while processing sperm samples for ART, of *M. pruriens* seed powder water extractions can be used as a viability enhancer. Hence, a clinical trial should be conducted to compare *M. pruriens* seed powder water extractions with commercially available semen preparation mediums.

Limitations

Since there was no improvement in sperm viability observed with *M. pruriens* ethanol extractions in fertile semen samples, semen samples collected from sub-fertile men were not tested with ethanol extractions.

Conclusions

M. pruriens distilled water extractions enhanced sperm viability and showed the best ability of preserving sperm viability up to 45 minutes. Therefore *M. pruriens* distilled water extractions can be considered as a favourable sperm viability enhancer in processing sperm samples for ART.

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