

Effect of Sri Lankan high grown black tea (*Camellia sinensis* L. O. Kuntze) on motility of human spermatozoa *in vitro*

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ABSTRACT

This study examined the effects of black tea brew (BTB) on motility of human spermatozoa *in vitro*. This was done using Sri Lankan high grown Dust grade No:1 black tea and freshly ejaculated human semen. Different concentrations (250, 500, or 1000 mg/kg) of BTB was made in isotonic saline (0.9% NaCl, w/v) using freeze dried tea samples. These were mixed with equal volumes of fresh semen adjusted to have a density of 20×10^6 and motility assessments were made at +5, +15, +30, and +60 min of incubation at 37 °C using WHO criteria. The results showed that BTB did not change the percentage motility of spermatozoa significantly ($P > 0.05$). However, BTB apparently induced a marked increase in the lateral head displacement of spermatozoa. In addition, BTB was non toxic to sperm. It is suggested that Sri Lankan black tea may be beneficial for male fertility.

Key words: *Camellia sinensis*, Sri Lankan tea, black tea, Dust No: 1, sperm motility, human sperm

INTRODUCTION

The typical black tea brew (BTB) processed from tender shoots of the perennial shrub *Camellia sinensis* L. O. Kuntze (Family: Theaceae) is used as a regularly consumed beverage in many communities in the world. Infact, it is the most consumed beverage of the world besides water (Modder and Amarakoon, 2002). A typical BTB contains 0.35% tea solids in water. This includes a variety of phytochemical constituents including caffeine, theophylline, flavonoids, and fluoride in an easily assimilable form (Balentine et al., 1997; Modder and Amarakoon, 2002). These four constituents are known to affect sperm function (Haesungcharern and Chvlavantnatol, 1973; Levin et al., 1981; Aitken et al., 1983; Schoff and Lardy, 1987; Loughlin and Agarwal, 1992).

Caffeine is a well known sperm motility stimulant (Haesungcharern and Chvlavantnatol, 1973; Levin et al., 1981; Aitken et al., 1983). Evidence is also present to show theophylline enhance sperm motility (Loughlin and Agarwal, 1992). Flavonoids are strong antioxidants scavenging reactive oxygen species (ROS) and free radicals

(Modder and Amarakoon, 2002) which are detrimental to sperm motility (Naz and Minhas, 1995; Baker et al., 1996; Griveau and Le Lannov, 1997). Further, a variety of antioxidants have shown to improve sperm motility (Naz and Minhas, 1995; Baker et al., 1996; Griveau and Le Lannov, 1997). Fluoride on the other hand is known to impair sperm motility (Schoff and Lardy, 1987). These findings suggest that tea may influence sperm motility, but this has not been investigated hitherto.

The aim of this study was to examine the effect of black tea on motility of human spermatozoa. This was investigated *in vitro* with freshly ejaculated semen and Sri Lankan high grown Dust grade No: 1 black tea. Dust grade tea was selected as it is the most widely consumed type of tea by the Sri Lankan tea drinkers.

MATERIALS AND METHODS

Dust grade No : 1 black tea that was manufactured at St Coombs estate tea factory (1362 m above mean sea level) of the Tea Research Institute, Talawakelle, Sri Lanka with green leaves from the same estate using orthodox-rotovane manufacture technique. Black tea brew (BTB) was made according to the ISO standards (Anon, 1980): 2g in 100 ml boiling water and brewed for 5 min. This was then freeze dried and stored at 4 °C in brown colored airtight bottles. The freeze dried samples were dissolved in isotonic saline (0.9% NaCl w/v) at pH 5.5 to make three different concentrations (2000; 1000 and 500 mg/ml) so that the final concentration after mixing with semen become 1000, 500 and 250 mg/ml.

Fresh semen samples were obtained from healthy adult volunteer donors (25-27 years). Prior to collection of samples all the donors were informed of the World Health Organization (WHO) guidelines on semen collection (WHO 1999). Following liquefaction, semen analysis was performed as per WHO criteria (WHO 1999). All the ejaculates selected (n = 12) had a volume ≥ 1 ml, a sperm concentration of $\geq 25 \times 10^6$ /ml, motility of $\geq 40\%$ and normal morphology of $\geq 60\%$. Spermatozoa were suspended in isotonic saline in order to obtain a final concentration of 20×10^6 /ml for the evaluation of effect of BTB on motility.

Spermatozoal suspensions (100 μ l) were placed in Falcon tubes. An equal volume of saline (control) or BTB in saline was added, mixed well and incubated at 37 °C in an atmosphere of 5% CO₂ in air. Aliquots of 10 μ l were transferred onto separate clean glass slides at 37 °C and covered with a cover glass (22 x 22 mm). The percentage of motile spermatozoa belonging to the grades a, b, c and d were assessed at each concentration at +5, +15, +30 and +60 min at a magnification of x 400 under phase contrast optics using an eyepiece graticule. At least 100 cells were counted in each

concentration at different time points. In addition, spermatozoa exhibiting enhanced lateral head displacement was noted. Sperm motility assessments were made by two experienced investigators who were blind folded to the treatments.

The results are expressed as mean \pm SEM. Statistical comparisons were made using ANOVA followed by Duncan's new multiple range test. Significance was set at $P < 0.05$.

RESULTS

The results obtained are summarized in Table 1. As shown, BTB did not induce any significant ($P > 0.05$) change either in the overall percentage motility or percentage motility of different individual grades (a, b, c and d). However, a marked increase in the amplitude of the lateral head displacement of spermatozoa treated with BTB was evident, at all three concentrations tested. The percentage number of spermatozoa exhibiting such movement characteristics was $> 75\%$. Further, BTB did not cause any agglutination of spermatozoa.

DISCUSSION

In this study we examined the effect of BTB on human sperm motility using an inexpensive, simple but reliable *in vitro* technique. Subjective nature of assessment of sperm motility is the main limitation of this procedure. However, *in vitro* studies are often used to investigate the effects of various agents on motility of sperm (Aitken et al., 1983; Ratnasooriya, 1990; Ratnasooriya et al., 1991; Ratnasooriya and Premakumara, 1992; Seneviratne et al., 1992; Naz and Minhas, 1995). The results showed that BTB had no effect on percentage sperm motility; neither increased nor reduced percentage motility of human sperm. Albeit BTB induced a marked enhancement in the amplitude of the lateral head displacement (assessed qualitatively). Depending on the experimental setting, both alterations in sperm motion characteristics (amplitude of lateral head displacement, linear velocity of progression frequency of head rotation) with no increment in percentage sperm motility (Naz and Minhas, 1995) and increase in percentage motility with no changes in sperm movement characteristics (Ratnasooriya, 1990) are reported with sperm motility stimulants.

Inability of BTB, in the present study to enhance percentage motility of human spermatozoa is surprising because it contains appreciable amount of caffeine and other methylxanthines (Balentine et al., 1997; Modder and Amarakoon, 2002) which are potent sperm motility stimulants (Haesungcharern and Chvlavantnatol, 1973; Levin et al., 1981; Aitken et al., 1983) and flavonoids with strong antioxidant activity (Modder and Amarakoon, 2002). However, BTB also contains fairly large quantities of fluorides

which are powerful sperm motility inhibitors (Schoff and Lardy, 1987). It is possible that these two diagonally opposing effects could be counter balancing each other thus excreting no net effect on percentage sperm motility. However, BTB managed to increase the amplitude of lateral head displacement. Although sperm motility in freshly ejaculated semen is often accepted as a reliable index of their potential fertilization ability (Vancerven et al., 1986) it is now known that the sperm movement characteristic that correlated highly with fertility is the velocity of forward progression and lateral head displacement (Aitken, 1986). Thus, the ability of BTB to increase lateral head displacement of sperm suggests that black tea may be beneficial to male fertility. However, obviously, further indepth studies involving both *in vivo* and *in vitro* studies are needed before firm conclusions are made. Human spermatozoa are extremely susceptible to toxicants (Seibert et al., 1989; Aitken et al., 1998). The fact that there was no significant increase in sperms exhibiting 'd' grade motility (immobilized) with incubation with BTB (up to 60 min) suggests that black tea is nontoxic to human sperm.

In conclusion, this study shows, for the first time that Sri Lankan high grown Dust grade No: 1 black tea is non toxic to human sperm and promotes lateral head displacement of sperm without influencing overall percentage motility *in vitro*.

ACKNOWLEDGEMENT

This investigation received financial support from the National Science Foundation of Sri Lanka under Grant No NSF/Fellow/2005/01. Thanks are also due to Mr. K. G. N. M. Gunaratne for technical assistance.

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Table 1. Effect of Sri Lankan high grown Dust grade No: 1 black tea brew (BTB) on human sperm motility *in vitro* (Mean \pm SEM)

| Treatment | Incubation Period | | | | | | | | | | | | | | | |
|------------|------------------------|-------|-------|-------|--------|-------|-------|-------|--------|-------|-------|-------|--------|-------|-------|-------|
| | 5 min | | | | 15 min | | | | 30 min | | | | 60 min | | | |
| | Grades of motility (%) | | | | | | | | | | | | | | | |
| | a | b | c | d | a | b | c | d | a | b | c | d | a | b | c | d |
| Control | 1.32 | 40.05 | 18.91 | 39.72 | 0.97 | 37.79 | 20.26 | 40.98 | 0.44 | 39.60 | 18.97 | 40.98 | 0.44 | 39.35 | 18.89 | 41.31 |
| (saline) | \pm | \pm | \pm | \pm | \pm | \pm | \pm | \pm | \pm | \pm | \pm | \pm | \pm | \pm | \pm | \pm |
| BTB | 0.62 | 1.68 | 1.94 | 1.87 | 0.29 | 2.69 | 2.08 | 2.04 | 0.22 | 1.08 | 1.02 | 1.25 | 0.22 | 1.56 | 2.36 | 2.56 |
| 250 mg/ml | 1.67 | 39.96 | 16.27 | 42.10 | 1.00 | 38.29 | 25.79 | 34.92 | 1.42 | 43.16 | 22.08 | 33.33 | 1.92 | 39.50 | 26.75 | 31.83 |
| | \pm | \pm | \pm | \pm | \pm | \pm | \pm | \pm | \pm | \pm | \pm | \pm | \pm | \pm | \pm | \pm |
| | 0.44 | 1.52 | 1.42 | 1.25 | 0.25 | 2.17 | 1.42 | 2.97 | 0.48 | 3.52 | 1.82 | 2.39 | 0.51 | 2.90 | 1.50 | 1.84 |
| 500 mg/ml | 1.09 | 36.29 | 29.61 | 33.00 | 1.02 | 35.26 | 32.57 | 31.15 | 2.70 | 40.22 | 23.48 | 33.59 | 2.21 | 38.33 | 27.55 | 31.90 |
| | \pm | \pm | \pm | \pm | \pm | \pm | \pm | \pm | \pm | \pm | \pm | \pm | \pm | \pm | \pm | \pm |
| | 0.29 | 0.61 | 2.52 | 2.71 | 0.28 | 1.46 | 3.12 | 1.83 | 0.57 | 2.42 | 3.01 | 2.69 | 0.48 | 1.32 | 1.99 | 1.41 |
| 1000 mg/ml | 1.38 | 37.22 | 21.99 | 39.41 | 2.06 | 39.75 | 24.32 | 33.87 | 0.85 | 38.72 | 26.40 | 34.02 | 0.72 | 40.32 | 24.21 | 34.75 |
| | \pm | \pm | \pm | \pm | \pm | \pm | \pm | \pm | \pm | \pm | \pm | \pm | \pm | \pm | \pm | \pm |
| | 0.43 | 1.58 | 1.51 | 1.86 | 0.53 | 1.27 | 1.17 | 1.96 | 0.26 | 1.79 | 3.76 | 3.07 | 0.24 | 1.77 | 1.88 | 2.32 |