

## Role of Alcoholic Turmeric (*Curcuma longa*) Extract in Outcome of *in vitro* Sperm Activation for Infertile Patients

Muhammad-Baqir MR Fakhridin *PhD*

Dept. of Clinical Reproductive Physiology, Institute of Embryo Research and Infertility Treatment, Al-Nahrain University

### Abstract

- Background** Semen samples are prepared for assisted reproduction by selecting a population of highly motile, morphologically normal sperm and removing the seminal plasma, leukocytes and bacteria. Culture media provide the spermatozoa with needs that maintain optimal function of spermatozoa to give rise excellent results during semen preparation.
- Objective** The aim of this study was to investigate the effect of alcoholic extraction of turmeric (AET) on sperm parameters during *in vitro* sperm activation (ISA) for asthenozoospermic (AZ) and oligoasthenozoospermic (OAZ) patients.
- Methods** Seventy four infertile patients were included, and classified into two groups according to their sperm parameters. Semen sample was divided into 3 aliquots. One mL of Earl's medium either alone (control group) or supplied with one concentration of AET (5 µg/mL or 10 µg/mL) was over layered the pellet, and the three tubes were incubated at 37 °C for 30 min in air incubator. Sperm concentration, motility, grades activity, progressive motility, normal morphology and agglutination were assessed pre- and post-activation *in vitro*.
- Results** Results revealed an enhancement of most sperm parameters for control and both treated groups post-activation as compared to pre-activation. Post-ISA, sperm parameters for both treated groups were better than the control group. However, best results for improvement of sperm parameters were assessed within treated group (5 µg/mL of AET).
- Conclusions** The lower concentration of alcoholic turmeric extraction enhanced human sperm parameters during ISA without any harmful effects on sperm physiology. The results are also useful as a guide for further standardization of turmeric extracts used for pharmaceutical purposes in the techniques of assisted reproduction.
- Key words** Male infertility, Sperm activation, Turmeric.

### Introduction

Thousands of chemical structures have been identified in plant foods that have complementary and overlapping actions, including antioxidant effects, modulation of detoxification enzymes, stimulation of the immune system, reduction of inflammation, modulation of metabolism and antimicrobial

effects <sup>(1)</sup>. Turmeric is a spice that comes from the root *Curcuma longa*, a member of the ginger family, Zingiberaceae <sup>(2,3)</sup>. Turmeric contains protein (6.3%), fat (5.1%), minerals (3.5%), carbohydrates (69.4%) and moisture (13.1%) <sup>(4)</sup>. Therefore spice turmeric, an important ingredient in the food preparation,

blocks the formation of hazardous Maillard reaction products and its mutagenic activity<sup>(5)</sup>. Turmeric has been used for its medicinal properties for various indications and through different routes of administration, including topical, oral, and inhalation<sup>(2)</sup>. Moreover, curcumin, the active principle isolated from turmeric, exhibits antimutagenic and anticarcinogenic activity<sup>(5)</sup>. The active principles of turmeric have been evidenced in several animal studies to exert hypolipidemic and antioxidant properties<sup>(6)</sup>. However, curcumin have been reported to interfere with inflammatory processes<sup>(1)</sup>.

The dietary turmeric is an effective anti-mutagen and it may be useful in chemoprevention against cancers of different organs<sup>(7)</sup>. The potential efficacy of fresh turmeric paste to heal wounds was tested in a preclinical study in an animal model<sup>(8)</sup>. Some research suggests that as an antioxidant, turmeric may help in the prevention of conditions such as cancer and heart disease. It was reported that the turmeric may lower levels of low-density lipoprotein cholesterol ("bad cholesterol") and total cholesterol in the blood<sup>(9)</sup>. Also, turmeric may lower blood sugar and may have additive effects with diabetes medications, and increased sperm count and motility. In addition, turmeric may stimulate contractions of the uterus and may alter menstrual periods<sup>(10,11)</sup>.

The development of assisted reproductive techniques (ARTs) as treatment modalities for infertility during the last two decades has led to the development of a wide range of different sperm preparation methods<sup>(12)</sup> and culture media<sup>(13)</sup>. Initially, different media were generated, and contained the same components, yet each medium was characterized by having a different component at a high concentration<sup>(14)</sup>. However, sperm isolation procedures involve the minimum of physical trauma because the shearing forces associated with centrifugation stimulate reactive oxygen species (ROS) generation in human semen samples<sup>(15)</sup>. Spermatozoa are

very vulnerable to oxidative stress by virtue of their high cellular content of unsaturated fatty acids and limited protection by cytoplasmic antioxidant enzymes<sup>(16)</sup>. Therefore, the aim of the present study was to investigate the effect of alcoholic turmeric extraction (AET) on human sperm parameters during *in vitro* activation for asthenozoospermic and oligoasthenozoospermic patients.

## Methods

### 1. Infertile patients:

This study was conducted at Institute of Embryo Research and Infertility Treatment, Al-Nahrain University, Baghdad, IRAQ. In the present study, seventy four infertile patients were involved, and classified into two major groups according to parameters of spermatozoa involving asthenozoospermic (AZ, no. 28) and oligoasthenozoospermic (OAZ, no. 46) patients. Complete history and physical examination for infertile patients were achieved before starting this study.

### 2. Seminal fluid analysis (SFA):

The semen sample was collected after 3-5 days of abstinence directly into a clean, sterile, wide open Petri dish by masturbation in a room near to the laboratory. The containers were labeled with name and age of patients, abstinence period and time of collection. According to manual of WHO<sup>(17)</sup>, SFA was done and involved macroscopic and microscopic examinations as mentioned in details by Fakhrildin<sup>(18)</sup>.

### 3. Preparation of alcoholic turmeric extract:

High quality of local Turmeric was purchased. Pieces of turmeric were further cleaned from dusts and crushed into turmeric powder using electrical mixer. 10 g of turmeric powder were added to 100 mL of 90% ethanol in a clean glass beaker, and the mixture was stirred for 4 hours period, then the mixture was filtered using Whattman filter paper to collect alcoholic extraction of turmeric (AET) in another clean glass beaker<sup>(6,19)</sup>. AET was divided into 5 parts in wide open glass Petri dishes for evaporation

of ethanol throughout one night in the laboratory, and using evaporator for complete evaporation. Finally, the residual of AET, as dried material, was weighted and stored in dark universal glass tube for further use. Then, 0.5 mg and 1 mg of dried AET were dissolved in 100 mL of Earl's medium to prepare 5 µg/mL and 10 µg/mL of AET, respectively.

#### 4. *In vitro* sperm activation (ISA):

One mL of liquefied semen sample was placed inside Falcone conical tube and mixed with 0.5 mL of Earl's medium, then; suspension was centrifuged at 2250 rpm for 6 minutes at room temperature. The supernatant was discarded, and the pellet was overlaid with 1 mL of Earl's medium either alone (control group) or enriched with two concentrations (T1; 5 µg/mL and T2; 10 µg/mL) of alcoholic turmeric extract. During *in vitro* sperm activation, three tubes were incubated at 37°C for 30 minutes. Thereafter, sperm parameters were examined in the three groups.

#### 5. Experimental design:

This study was designed to investigate the effects of alcoholic turmeric extraction on outcome of ISA for asthenozoospermic and oligoasthenozoospermic semen samples. Therefore, examinations of SFA as pre-activation group at start, and other three groups of control and two treated (T1; 5 µg/mL and T2; 10 µg/mL) groups as post-activation groups. Examination of sperm parameters involves sperm concentration, motility (%), grades activity (%), normal morphology (%) and agglutination (%).

#### 6. Statistical analysis:

All values were expressed as Mean  $\pm$  S.E.M. Statistical Package for Social Studies (SPSS; version 14) was used for statistical analysis.

Depending on experimental design and groups of this study, paired *t*-test and two way ANOVA test were applied to compare between groups of pre- and post-activation, and to compare among control and post-activation groups. P value at  $< 0.05$  was considered as statistically significant.

#### Results

Results of the present study for asthenozoospermic patients showed that the percentages of sperm motility and progressive sperm motility were decreased as compared to standard criteria of WHO (1999). Meanwhile, percentage of sperm agglutination was higher when compared to normal WHO criteria 1999 (Table 1).

Sperm concentration, non-progressive sperm motility (%), immotile sperm (%) and sperm agglutination (%) for control and treated groups were significantly decreased ( $P < 0.01$ ) in the post-activation as compared to the pre-activation (Table 1).

In contrast, percentages of sperm motility, progressive motile sperm and normal sperm morphology for three groups were increased significantly ( $P < 0.01$ ) in the post-activation when compared to pre-activation (Table 1).

Among control and both treated groups, non significant differences ( $P > 0.05$ ) were observed in the sperm concentration, non-progressive motile sperm (%), normal sperm morphology (%) and sperm agglutination (%) as presented in table 1.

From the same table, percentages of sperm motility and progressive motile sperm for T1 group were significantly increased ( $P < 0.05$ ) as compared to the control and T2 group. However, significant reduction ( $P < 0.05$ ) in the percentage of immotile sperm for T1 group when compared to the control and T2 groups (Table 1).

Table 1. Parameters of *in vitro* pre- and post-activated sperm using Earl's medium enriched with two concentrations of alcoholic turmeric extract for asthenozoospermic patients

Sperm parameters		Asthenozoospermic semen samples No = 28				WHO, 1999 criteria
		Pre- activation	Post-activation			
			Control	T1; 5 mg/mL	T2; 10 mg/mL	
Sperm concentration		54.68±2.73	15.72±1.98	20.84±1.83	18.08±1.94	20X 10 <sup>6</sup> sperm/mL ≥ 50 %
Sperm Motility (%)		38.76±2.42	78.66±2.81	88.29±2.63 <sup>†</sup>	80.21±2.17	
Sperm grade activity (%)	PMS	29.55±1.74	75.61±1.89	86.26±2.48 <sup>††</sup>	77.78±2.67	≥ 50 % of grades (A+B)
	NPMS	9.24±1.46	2.04±0.83	2.09±0.64	2.41±0.72	
	IS	61.23±1.38	21.35±1.13	11.68±0.81 <sup>††</sup>	19.80±0.87	
Normal sperm morphology (%)		46.27±1.75	83.56±2.37	85.12±2.71	84.29±2.79	≥ 30 %
Sperm agglutination (%)		12.43±1.23	-	-	-	< 10 %

PMS = Progressive motile sperm, NPMS = Non-progressive motile sperm, IS = immotile sperm, \* = P<0.01 as compared to three groups of post-activation, † = P<0.01 as compared to the control group of post-activation, ‡ = P<0.05 as compared to another treated group of post-activation.

Table (2) shows sperm parameters for oligoasthenozoospermic patients complaining from reduction in the sperm concentration and motility (%) when compared to normal criteria of WHO (1999). As compared to pre-activation *in vitro*, there were significant reductions (P<0.01) in sperm concentration, immotile sperm (%) and sperm agglutination (%) in the post-activation of three groups. Conversely, percentages of sperm motility, progressive motile sperm and normal sperm morphology for control and both treated groups were significantly increased (P<0.01) in the post-activation as compared to pre-activation (Table 2).

From the same table, non significant differences (P>0.05) were noticed in the sperm concentration, normal sperm morphology (%) and sperm agglutination (%) in the post-activation against the control and both treated groups. However, percentages of sperm motility and progressive motile sperm for T1 group were significantly increased (P<0.05) as compared to the control group. In contrast, significant reduction (P<0.01) was assessed in the immotile sperm (%) for T1 group when compared to the control group. Post-activation *in vitro* caused a significant reduction (P<0.05) in non-progressive motile sperm (%) of control and T1 groups as compared to T2 group.

Table 2. Parameters of *in vitro* pre- and post-activated sperm using Earl's medium enriched with two concentrations of alcoholic turmeric extract for oligoasthenozoospermic patients

Sperm parameters		Asthenozoospermic semen samples No = 28				WHO, 1999 criteria
		Pre- activation	Post-activation			
			Control	T1; 5 mg/mL	T2; 10 mg/mL	
Sperm concentration		17.26±1.39	6.82±0.82	8.68±1.07	8.13±1.16	20X 10 <sup>6</sup> perm/mL ≥ 50 %
Sperm Motility (%)		35.92±1.72	76.79±2.28	87.23±2.17 <sup>†</sup>	83.72±2.03	
Sperm grade activity (%)	PMS	26.58±1.52	72.18±1.84	79.37±1.81 <sup>†</sup>	74.50±1.93	≥ 50 % of grades (A+B)
	NPMS	9.32±0.73	4.61±0.47	5.86±0.64	9.22±0.81 <sup>††</sup>	
	IS	64.09±1.52	23.22±1.17	12.76±0.45 <sup>†</sup>	16.29±0.73	
Normal sperm morphology (%)		36.81±1.31	81.46±1.73	83.87±1.79	83.27±1.58	≥ 30 %
Sperm agglutination (%)		6.67±0.74	-	-	-	< 10 %

PMS = Progressive motile sperm, NPMS = Non-progressive motile sperm, IS = immotile sperm, \* = P<0.01 as compared to three groups of post-activation, † = P<0.01 as compared to the control group of post-activation, ‡ = P<0.05 as compared to another treated group of post-activation.

## Discussion

After *in vitro* sperm activation (ISA), all sperm parameters for asthenozoospermic (AZ) and oligoasthenozoospermic (OAZ) patients were enhanced in the control and both treated groups, except for sperm concentration. The reduction in the sperm concentration results from swim-up of only active motile sperm during ISA<sup>(20)</sup>. However, most sperm motility parameters and sperm progressive motility (%) especially were improved in both AET-treated groups better than the control group. In this study, we used one centrifugation step because the original sperm parameters for both groups of infertile patients were not severe cases. However, strategies involving repeated high speed centrifugation are also occasionally used in an effort to harvest as many cells as possible from the ejaculates of severely OAZ patients<sup>(21)</sup>. In addition, it has been demonstrated that the method employed for preparing spermatozoa influences ROS production by human sperm suspensions and this inversely correlates with the fertilizing potential of the spermatozoa *in vitro*<sup>(22)</sup>.

Numerous studies have indicated that the AET improves  $\text{Ca}^{2+}$ -transport to correct the defective  $\text{Ca}^{2+}$  homeostasis<sup>(4)</sup>, and reduces cholesterol content<sup>(23,24)</sup>. It was reported that the reduction of cholesterol is combined with an increase of  $\alpha$ -tocopherol level in rat plasma, suggesting *in vivo* interaction between curcumin and  $\alpha$ -tocopherol that may increase the bioavailability of vitamin E and decrease cholesterol levels<sup>(25)</sup>. Turmeric has many active components, but curcumin is the most potent ingredient. It is a powerful anti-inflammatory and anti-oxidant and has greater effects in preventing free radical damage, compared with vitamins C, E and superoxide dismutase<sup>(26)</sup>. However, modulation in  $\text{Ca}^{2+}$  and reduction of cholesterol are important prerequisites for sperm hyperactivation, capacitation, acrosomal reaction and fertilization<sup>(27,28)</sup>.

Under *in vivo* conditions, potentially fertile spermatozoa are separated from immotile spermatozoa, debris and seminal plasma in the

female genital tract by active migration through the cervical mucus. During this process, only progressively motile spermatozoa are selected<sup>(20)</sup>. Researchers were able to show that morphologically abnormal sperm with low motility produced the highest levels of ROS<sup>(29)</sup>. Also, the results of experiments suggest that abnormal sperm with low motility produce high levels of ROS which can then subsequently damage and affect the motility of otherwise normal sperm within the semen population<sup>(30)</sup>. Actually, as soon as seminal plasma is removed, the spermatozoa become vulnerable to free radical attack by contaminating leukocytes and both sperm function and DNA integrity can be compromised<sup>(21)</sup>. In addition, harmful effect of centrifugation process during sperm preparation<sup>(31)</sup>. For this reason, AET was used. Also, AET lowers reactive oxygen species (ROS) generated by centrifugation during ISA through antioxidant activity<sup>(32,33)</sup>. Spermatozoa are rich in polyunsaturated fatty acids and more liable for lipid peroxidation by ROS<sup>(23)</sup>. ROS and their derivatives can damage various biomolecules including lipids, proteins and DNA. There is evidence suggesting that free radicals and ROS play a significant role in many cases of male infertility<sup>(30)</sup>. Curcumin, the active ingredient of the turmeric, is a strong antioxidant, and reportedly several times more potent than vitamin E as a free radical scavenger [1]. *In vitro*, curcumin can significantly inhibit the generation of ROS like superoxide anions,  $\text{H}_2\text{O}_2$  and nitrite radical generation by activated macrophages<sup>(34)</sup>. This is brought about by maintaining the activities of antioxidant enzymes like superoxide dismutase, catalase and glutathione peroxidase<sup>(35)</sup>. Curcumin binds to lipoxygenase in a noncompetitive manner<sup>(36)</sup>.

In contrast, it was reported that curcumin inhibits human sperm motility and has the potential for the development of intravaginal contraception<sup>(36)</sup>. These harmful effects of curcumin on sperm motility may be indirectly through physiological and biochemical effects



on vagina and its lining epithelium by increasing NO which has negative activity on sperm motility<sup>(23)</sup>.

From the results of the present study, it can be concluded that the low concentrations of alcoholic turmeric extract enhanced human sperm parameters during ISA without any harmful effects on sperm physiology. The results are also useful as a guide for further standardization of turmeric extracts used for pharmaceutical purposes in the techniques of assisted reproduction.

### Acknowledgement

The author is grateful to Assist. Lecturer Usama IM Al-Zubaidi, Applied Embryology Dept. at Institute of Embryo Research and Infertility Treatment, Al-Nahrain University, Baghdad, IRAQ for his valuable assistance throughout this work.

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Correspondence to: Dr. Muhammad-Baqir Fakhrildin,  
E-mail: [art\\_mbmrfd@yahoo.com](mailto:art_mbmrfd@yahoo.com).  
Received 26<sup>th</sup> Apr. 2011; Accepted 19<sup>th</sup> Jun. 2011