Research Article

Effect of Alcohol Extract of *Prunus avium* on *In vitro* Sperm Activation of Human Semen Samples

Mufedah Ali Jawad[1], Amal Abdul Wahid Mohammed[1], Hayder B Sahib[2]*, Muayad Sraibet Abbood[2], Wathiq Qutaiba Shakir[1]
[1]High Institute for Infertility Diagnosis and ART Al-Nahrain University.
[2]Department of Pharmacology, College of Medicine, Al- Nahrain University.
***Corresponding author's E-mail:** haider_bahaa@yahoo.com

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ABSTRACT

The Wild cherry, Sweet cherry (botanic name, *Prunus avium*) is a species of cherry fruits which has wide distribution in the world. To evaluate the efficacy of using global medium alone versus global medium plus alcohol extract of *Prunus avium* for *in vitro* sperm activation (ISA) to asthenozoospermic human semen samples to improve sperm parameters. The study was done on thirty five asthenozoospermic human semen samples. The semen parameters were recorded according to WHO criteria (1999). All semen samples were undergone activation, by using Global medium alone as control group, and by using a mixture of global medium with ethanol extract of *Prunus avium* to be considered as an experimental group. The results were recorded 30 minutes and 60 minutes after incubation. The results of this study showed significant difference ($P<0.05$) in percentage of sperm motility, grade A, grade B and percentage of normal sperm morphology of post activation with Global medium compared with pre-activation results of semen samples, there were significant difference ($P<0.05$) in these results between the experimental group and control group 30 and 60 minutes after activation. Mixing of alcohol extract of *Prunus avium* with sperm activation media has enhancing effect on sperm parameters.

Keywords: Antioxidant, *Prunus avium*, Sperm activation, Sperm parameters.

INTRODUCTION

Since ancient times, herbs and spices have been recognized to possess biological activities, including antibacterial, antifungal and antioxidant properties. Considering this, some medicinal plants and spices have been extensively studied for their antioxidant activity and radical scavenging properties in the past several years.[1-3] Many of the stone fruits like sweet cherry (*Prunus avium* L.) have been cultivated since ancient times.[4] Anatolia is the origin of sweet cherry, like many other fruits.[5] Cherries are members of the *Rosaceae* family, and subfamily *Prunoideae.*[6] It contains beside water, saccharides in which glucose was found to have the highest content, followed by fructose, sorbitol and sucrose[7], vitamins (A, B and C), pectin, organic acids mainly malic acid[8], also minerals mainly potassium, magnesium, phosphor and calcium.[9] In addition *Prunus Avium* has been found to contain melatonin- MLT (*N*-acetyl-5-methoxytryptamine) which is an endogenous hormone found to be present in all vertebrates pineal gland. It was reported to be a potent free radical scavenger and a broad-spectrum antioxidant.[10] In addition, Melatonin MLT detoxifies a variety of free radicals and reactive oxygen intermediates, including the hydroxyl radical, the peroxynitrite anion, singlet oxygen and nitric oxide.[11] Reactive oxygen species (ROS) are believed to be important mediators of damage to spermatozoa that have been associated with various indices of cellular injury.[12] Lipid peroxidation and DNA fragmentation have been correlated with exposure of sperm to ROS.[13, 14] In addition, excessive ROS formation by spermatozoa in an *in-vitro* condition has been associated with decreased motility, abnormal morphology and a lowered capacity for sperm- oocyte penetration [15,16], therefore the sperm preparation for ART including artificial insemination (AIH) is to maximize the chances of fertilization.[17] It is apparent that spermatozoa of mammalian species including human can acquire the ability to fertilize after a short incubation in defined culture media.[18] In the present study alcohol extract of *Prunus avium* was used in sperm activation procedure to get better results.

MATERIALS AND METHODS

Preparation of the solution

3.8 gm of alcohol extract of *Prunus avium* to be added to 8ml of global medium, after adjustment of its pH to be (7). 0.5ml been taken from the mixture and added to the semen sample to reach to 2ml then begins the swim up technique.

Procedure

Thirty five semen samples from asthenozoospermic male patient who visited the male infertility clinic in the High Institute of Infertility Diagnosis and ART/ Al-Nahrain University were included in this study. Semen parameters were recorded first according to manual of WHO criteria [19], and then activation was done with global medium by using swim up technique (control group). Results were recorded at 30 and 60 minutes after activation. A mixture of alcohol extract of *Prunus avium* with global medium was used to activate the same semen samples (experimental group) and results were recorded after 30

minutes and after 60 minutes. Comparison was done between results of control and experimental groups. All ethics Issues been approved by ethics committee/ College of Medicine/ Al –Nahrain University / Iraq.

RESULTS

Comparison of sperms parameters in pre and post activation with global medium

Table 1 showed significant increase in percentage of sperm motility, (P<0.05) forward progresses motility (grade A), on progresses motility (grade B) and percentage of normal sperm morphology in post-activation with global medium alone compared to pre-activation results as Comparison of sperm parameters in control group and experimental group 30 minutes after activation.

Table 2 showed the results of activation using global medium alone as control group and global medium with alcohol extract of *Prunus avium* as experimental group 30 minutes after activation and these results showed significant (P< 0.05) increase in the percentage of motile sperm, grade A, grade B, percentage of normal morphology in the experimental compared to control group.

Comparison of sperm parameters in control group and experimental group 60 minutes after activation

Table 3 showed the results of activation in the control group and experimental group 60 minutes after activation and these results showed significant (P< 0.05) increase in the percentage of motile sperm, grade A, grade B, percentage of normal morphology in the experimental compared to control group.

Table 1: Results of sperm parameters before and 30 minutes after activation with global medium

Sperm parameter	Concentration (million/mL)	Motility (%)	Grade of motility				Normal morphology (%)
			A (%)	B (%)	C (%)	D (%)	
Pre -activation	38.43±3.12	34.3±3.44	1.71±1.19	11.82±1.62	29.77±2.73	56.7±2.95	46.28±2.17
Post- activation	11.8±1.07	80.72*±3.92	23.14±4.46	38.28±3.52	19.3±2.72	19.28±3.19	73.14*±1.62

Table 2: Results of sperm parameters 30 minutes after activation with global medium alone (control group) and with mixture of global medium and alcohol extract of *Prunus Avium* (experimental group)

Normal Morphology (%)	Grade of activity				Motility (%)	Concentration (%)	Sperm parameters	
	D (%)	C (%)	B (%)	A (%)				
46.28±2.17	56.7±2.95	29.77±2.73	11.82±1.62	1.71±1.19	44.3±3.44	38.43±3.12	Pre-activation	
73.14±2.17	19.28±3.19	19.3±2.72	38.28±3.52	23.14±4.46	80.72±3.92	11.8±1.07	control	Post-activation
79.14*±3.51	5.61±1.60	17.42±3.56	39.17*±3.74	37.8*±4.62	94.36*±3.93	10.4±1.69	experimental	

Table 3: Results of sperm parameters 60 minutes after activation in the control group and experimental group

Normal morphology (%)	Grade of activity				Motility (%)	Concentration (Million/mL)	Sperm parameters	
	D (%)	C (%)	B (%)	A (%)				
46.28±2.17	56.7±2.95	29.77±2.73	11.82±1.62	1.71±1.19	34.3±3.44	38.43±3.12	Pre-activation	
53.14±1.61	20.23±3.18	11.4±2.72	41.23±3.51	27.14±4.46	79.77±4.92	11.2±1.07	control	Post-activation
62.14*±2.67	6.85±2.94	3.1±1.52	49.2*±3.73	40.85*±4.01	93.15*±4.03	10.4±1.67	experimental	

DISCUSSION

Prunus avium has been reported to contain various compounds which are known to have antioxidants, cyclooxygenase inhibitory activities, like anthocyanins and phenolics[20,21] which may provide protection from DNA cleavage, anti-inflammatory activity, lipid peroxidation, and membrane strengthening.[22,23] The result of the present study showed significant increase in the percentage of motile sperms, grade A, grade B and percentage of normal sperm morphology after using swim up technique in global medium compared to sperm parameters before activation. These results agree with previous studies which reported that the defined culture medium (CM) sometimes enriched with protein source and/or sperm stimulator.[24] Improvement of sperm motility and grade of activity was obtained as a result of special basic components of CM.[25] The results of post activation using alcohol extract of *Prunus avium* with global medium also showed significant increase in percentage of motility, grade A, grade B and normal sperm morphology compared to result of activation with global medium alone, this could be due to its chemical constituent of phenolics which act as anti oxidant. *Prunus avium* , has anthocyanins[26,27] and melatonin or N-acetyl-5-methoxytryptamine.[28] These antioxidants are compounds and reactions that dispose scavenge and suppress the formation of ROS or oppose their actions. Many studies have demonstrated that the effects of antioxidants may be because of its oxygen free radical defense mechanisms.[29-31] The high levels of antioxidants in

seminal plasma carried out an important role for spermatozoa *In vitro*. Lopes *et al*. (1998) demonstrated that addition of antioxidants significantly decreased the amount of spermatozoal DNA damage induced by ROS *in vitro* during incubation at 37°C.[14] The results indicate that supplementation of antioxidants by addition of alcohol extract of *Prunus avium* to sperm activation medium (global medium) to semen extender might be needed to improve the human semen quality for ART.

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