Effect of Alcohol on Human Sperm Parameters in vitro

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Abstract

This study aimed to investigate if the deleterious effect of alcohol exerted directly or mediated through metabolites so we added different ethanol concentrations (25,50 and100)mM to normal human spermatozoa in vitro. Three incubation periods (30, 45 and 60) minutes were used in this study .

The addition of different ethanol concentrations to glucosaline media which used in incubation of normal spermatozoa in vitro caused a significant decrease in sperm motility percentage and grade activity of the sperm in different incubation periods in exception the concentration 25 mM of ethanol at 30 minute of incubation period was caused a significant decrease in the sperm motility percentage only . This result refered to that the effect of ethanol on human spermatozoa was being directly without any mediator metabolite.

Key Words: Alcohol, Normal spermatozoa, male infertility

تأثير الكحول على معالم النطف البشرية في الزجاج

الخلاصة

هدفت الدراسة الحالية لمعرفة التأثيرات الضارة للكحول كونها تظهر مباشرة أو من خلال ناتج تأيض الكحول استخدمت تراكيز مختلفة من الايثانول (100,50,25) ملي مولاري إلى نطف بشرية سوية في الزجاج .تم استخدام ثلاث فترات حضن (60,45,30) دقيقة .

سبب إضافة التراكيز المختلفة من الايثانول إلى وسط المحلول الفسيولوجي المستخدم في حضن النطف البشرية السوية في الزجاج حدوث نقصان معنوي $P<0.05$ في النسبة المئوية للنطف المتحركة ودرجة نشاط النطف لفترات الحضن المختلفة ماعدا التركيز 25ملي مولاري وعند فترة حضن30 دقيقة فقط أدى إلى حدوث نقصان معنوي في النسبة المئوية للنطف المتحركة فقط .هذه النتيجة تشير إلى أن تأثير الايثانول على النطف البشرية يكون مباشر بدون أي متأيض وسطي .

الكلمات المفتاحية: الكحول، النطف السوية ، عدم الخصوبة لدى الذكور، معايير النطف

Introduction

Alcohol is one of the lifestyle factors that has effects on male reproduction . Human and animal studies have shown that alcohol consumption cause fertility disturbances through low sperm count, decrease normal morphology [1,2], low sperm motility [3], alter testicular and accessory gland morphology, cause reduction in reproductive organ weight and impaired epididymal sperm maturation [4]. In addition Martinez et al.[5] were noticed irregular diameter of seminiferous tubules and high amount of death cells in the lumen after chronic ethanol exposure . Alcohol has been correlated with decrease plasma testosterone level [6], hypogonadism, ejaculation problems and impotence [7]. Nervous system damage can occur via oxidative metabolite acetaldehyde [8]. To know that deleterious effects of alcohol on human spermatozoa exerted directly or mediated through metabolites, we added ethanol to media which used in the incubation of normal spermatozoa to eradicate any factors that occur in vivo and ethanol concentrations which

used in this study were selected on the basis of experimental evidence from study performed on other cellular model [9] which tested the effect of ethanol at (25, 50 and 100)mM on the frontal cortex synaptosomes and reported that these treatment induced signs of toxicity.

Materials and Method

Semen Samples

Semen samples were obtained from 10 healthy men aged (20 -35) years with normal semen parameters reffered to Center of infertility in Babylon Hospital for Delivery and Children . The criteria for participation in the study included the absence of anatomical problems such as varicocele or cryptochidism or genitourinary infections .

Semen collection and preparation

Samples were collected in the hospital by masturbation into a wide – mouthed sterile plastic container after 4 days of sexual abstinence and examined after compelet liquefaction for 20-30 minutes at 37 ºC . Semen analysis was performed according to WHO criteria [10].

Two ml of each semen sample was divided into four equal splits .putting separately in four centrifuged tubes. Each 0.5 ml of semen was mixed with 1 ml of glucosaline media supplemented with 20% inactive maternal serum . The mixture was centrifuged at 2000 rpm for 5 minutes ,the supernatant was discarded and the final pellet in four tubes were overlayered with glucosaline media the first tube with glucosaline alone considered as (control), glucosaline +25mMof ethanol, glucosaline +50 mM of ethanol and glucosaline +100 mM of ethanol are considered as treatments of ethanol. Aliquot of all analyzed specimens were incubated for (30,45 and 60) minutes at 37 ºC. adrop of top part of glucosaline alone and glucosaline with each ethanol concentration were aspirated by pipette and examined to evaluate sperm parameters which included :sperm concentration , sperm motility percent , grade activity and abnormal sperm morphology percent .

Statistical analysis were performed by using Completely Randomized Design (CRD) and lest Significant difference (LSD) to compare between ethanol treated and control sperm [11].

Results

The incubation of normal spermatozoa with glucosaline media which supplemented with different ethanol concentration (25, 50 and 100) mM lead to a significant decreas ($P<0.05$) the sperm motility percent and grade activity of the sperm incomparing with control (glucosaline alone) in all incubation periods
(30, 45 and 60) minutes exception 25 mM of ethanol at 30 minutes of incubation period which caused significant decrease($P<0.05$) in sperm motility percent only incomparing with control . Table (1,2 and 3).

Table 1 Effect of ethanol at different concentrations on human sperm parameters after 30 minutes of exposure in vitro.

Parameters	After centrifugation (mean ± SD)			
	Control	ethanol concentrations (mM)		
		25	50	100
Sperm concentration (x 10^6)	16.2 a ± 1.4	15.9 a ± 1.3	14.7 a ± 1.7	14.5 a ± 1.7
Sperm motility (%)	75 .0 a ± 1.9	60.0 b ± 4.5	38.0 c ± 7.8	18.0 d ± 3.8
Grade activity	3.6 a ± 0.1	3.3 a ± 0.1	2.5 b ± 0.3	1.5 c ± 0.2
Abnormal sperm morphology (%)	14.6 a ± 1.1	14.9 a ± 1.0	15.2 a ± 1.5	14.8 a ± 0.9

Different letters refers to significant difference.
P<0.05

The progressive decline in these sperm parameters by increasing the concentration of ethanol, this refer to dose dependent effect of ethanol on human sperm motility percentage and grade activity in all incubation periods.

Table 2 Effect of ethanol at different concentration on human sperm parameters after 45 minutes of exposure in vitro

Parameters	After centrifugation (mean ± SD)			
	Control	ethanol concentrations (mM)		
		25	50	100
Sperm concentration (x 10^6)	17.8 a ± 0.7	17.1 a ± 2.1	16.4 a ± 1.9	16.6 a ± 1.4
Sperm motility (%)	79.5 a ± 1.7	55.0 b ± 4.0	34.0 c ± 6.1	17.5 d ± 3.0
Grade activity	3.8 a ± 0.07	3.3 b ± 0.1	2.3 c ± 0.2	1.4 d ± 0.2
Abnormal sperm morphology(%)	14.5 a ± 1.7	15.0 a ± 1.4	14.5 a ± 1.3	15.6 a ± 0.8

Different letters refers to significant difference .
P<0.05

While sperm concentration and abnormal sperm morphology percent in all treatments were uneffected when different ethanol concentrations were added to glucosaline media incomparing with glucosaline alone (control) in all incubation periods Table (1,2 and 3).

Table 3 Effect of ethanol at different concentrations on human sperm parameters 60 minutes of exposure in vitro .

Parameters	After centrifugation (mean ± SD)			
	Control	ethanol concentrations (mM)		
		25	50	100
Sperm concentration (x 10^6)	17.2 a ± 0.9	15.6 a ± 1.0	15.1 a ± 1.4	16.4 a ± 1.3
Sperm motility (%)	83.0 a ± 1.5	48.5 b ± 3.7	32.5 c ± 5.8	17.0 d ± 3.2
Grade activity	4.2 a ± 0.1	0.3 b ± 0.08	2.2 c ± 0.2	1.3 d ± 0.2
Abnormal sperm morphology (%)	14.8 a ± 1.2	15.4 a ± 1.2	14.0 a ± 1.4	13.9 a ± 1.4

Different letters refers to significant difference .
P<0.05

Discussion

This study showed that the incubation of nomal human spermatozoa with glucosaline media which supplemented with (25,50,and 100) mM of ethanol caused a significant decrease ($p < 0.05$) in sperm motility percent and grade activity of the sperm for different incubation periods and alcohol had a dose _dependent effect on these sperm parameters Table (1,2 and 3).This results is agreement with Dehghan et al.[12] and Talebi et al.[3] which they have been show in animal that ethanol exposure leads to significant decrease in sperm motility. Another study show that ethanol was promoted reactive oxygen species (ROS) generation and reduced the levels of antioxidant [13]. It is well known that human sperm motility had inverse correlation with ROS[14], this may be one pathway by which alcohol produces decreasing in sperm motility in this study. Mitochondria damage and decreasing in adenosine triphosphate (ATP) production occurs after ethanol exposure [15], so the reduction in grade activity of the sperm by ethanol in this study may be due to alcohol - induced damage of mitochondria and decreased ATP production which is essential to strong sperm motility.

The results of this study did not show any significant alteration in

sperm concentration and abnormal sperm morphology percent and this result agree with another studies which they have been show in animals that consume ethanol leads to distrubs sperm motility but not effect on sperm morphology [3] and chronic ethanol intake by animals caused increase in levels of free radicals but these radicals were unable to induce lipid peroxidation [16].

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