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Effect of Aloe vera extracts on in vitro human sperm parameters for asthenozoospermic patients

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Abstract

Background: *Aloe vera* is an evergreen perennial plant. It is widely used in modern herbal practice. The clear gel contained within the leaf makes an excellent treatment for wounds, burns and other skin disorders, speeding up the rate of healing and reducing the risk of infection. *Aloe vera* is also taken internally in the treatment of chronic constipation, poor appetite, and digestive problems. Semen analysis is the cornerstone of the evaluation of male fertility. Routine semen analysis can many times allow a definitive diagnosis of infertility. Therefore, the typical measurements done in a semen analysis include: sperm concentration, sperm motility, progressive sperm motility, sperm morphology, and foreign cell contamination.

Objective:

The present investigation was carried out to study the effect of low dose of fresh Aloe vera leaf gel extracts on in vitro sperm parameters for asthenozoospermic patients.

Materials and Methods:

Thirty semen samples of asthenozoospermic patients were used in this study. 5 µL of Aloe vera extract was mixed with 1 ml of each sample, left in the incubator for one hour, and then the sperm parameters including concentration, motility, morphology, agglutination, viability, and HOS test were recorded.

Results:

Highly significant increase at ($P\leq 0.01$) was recorded in the sperm concentration, percentages of sperm motility, progressive motility grade A, and normal sperm morphology, as well as sperm viability and HOS test. While a highly significant decrease ($P\leq 0.01$) was record in the percentages of sperm grades C and D, agglutination, and number of round cells.

Conclusion:

Using low dose of fresh Aloe vera extract enhanced sperm parameters especially in relation to motility, morphology and viability.

Key words: Aloe vera, male infertility, sperm, asthenozoospermia

تأثير مستخلص نبات الصبار*Aloe vera* على معايير نطف الانسان في الزجاج للمرضى الذين يعانون من وهن النطف

الخلاصة

اجريت هذه الدراسة لمعرفة تاثير الجرع الواطئة من مستخلص نبات الصبار *Aloe vera* على معايير نطف الانسان في الزجاج للمرضى الذين يعانون من وهن النطف. شملت الدراسة ثلاثون عينة مني من مرضى وهن النطف وبعد اجراء تحليل السائل المنوي وفقا للمعايير القياسية لمنظمة الصحة العالمية (WHO, 2010)تمت اضافة 5 مايكروليتر من مستخلص نبات الصبار لواحد مل من السائل المنوي لكل عينة وتركت في الحاضنة لمدة ساعة ثم سجلت معايير النطف والتي شملت تركيز النطف والحركة والنسبة المئوية لشكلياء النطف السوية والتلازن واجراء فحص انتفاخ الغشاء البلازمي للنطفة وحيوية النطف. اظهرت النتائج زيادة معنوية عالية ($P\leq 0.01$) في معدل تركيز النطف والنسبة المئوية للحركة التقدمية (A) للنطف ونسبة الشكلياء السوية كذلك حيوية النطف كما سجل انخفاض معنوي عالي ($P\leq 0.01$) في النسبة المئوية للحركة غير التقدمية (C) والنطف غير المتحركة (D) والتلازن وعدد

الخلايا الدائرية. يمكن الاستنتاج من الدراسة الحالية بان اضافة جرع واطئة من مستخلص نبات الصبار الى عينات السائل المنوي للمرضى الذين يعانون من وهن النطف لمدة ساعة ادى الى تحسين في معالم ووظائف النطف الرئيسية.

Introduction:

Infertility affects 15% of couples around the world. Male factor accounts for 40-50% of infertility. Half of all infertile couples have a component of male infertility and almost 30% of these cases are caused by a male factor (Iammarrone et al. 2003). Male infertility contributes to half of all infertility problems, and it is the underlying solitary reason (McLachlan and de Kretser, 2001, Waters et al. 2006). The causes of male infertility include obstruction, variococele, infection, and exposure to toxins and radiation. Even with advancements in understanding of human reproductive physiology, up to 23% of male infertility is idiopathic. Male infertility also can be caused by various genetic lesions such as gross chromosomal aneuploidies, rearrangements, microdeletions, and single-gene defects. It affects not only on genes controlling the male germ line, but also on the network involved in male gonadal development and male somatic development (Hassun Filho et al. 2005).Defective sperm function has been identified as the largest defined cause of human infertility, accounting for about 27% of all couples attending infertility clinics (Hull, 1986). Understanding of the chemical nature of the damage to the sperm plasma membrane responsible for this refractory state has been advanced by numerous, independent studies suggesting a key role for lipid peroxidation in the etiology of male infertility (Aitken and Clarkson, 1988, Alvarez et al. 1987). The possibility that peroxidative damage to the sperm plasma membrane might be involved in those cases of infertility characterized by a failure to exhibit sperm-oocyte fusion was suggested by studies indicating an association between the appearances of such defects and the hyperactive production of reactive oxygen species by the spermatozoa (Aitken et al. 1989). Antioxidant actions of some natural compounds such as vitamins and minerals, polyphenols, and other non-nutrient compounds of plants, which inhibit generation of ROS, or which scavenge free radicals, are therefore believed to be beneficial for human health and fertility (Badami et al. 2003, Braca et al. 2002).The *Aloe vera* plant has been known and used for centuries for its health, beauty, medicinal and skin care properties. 2000 years ago, the Greek scientists regarded *Aloe vera* as the universal panacea. The Egyptians called *Aloe* "the plant of immortality". The Aloe vera plant has been used for various purposes in dermatology (Amar et al. 2008, Grindlay and Reynolds, 1986). There is over 300 Aloe species, which have been used in several cultures like: Greece, Egypt, India, Mexico, Japan and China (Marshall. 1990). Studies on Aloe vera have largely upheld the therapeutic claims of anti-diabetic, anti-cancer and antibiotic properties of this plant extract (Kosif et al. 2008). A study by (Atherton, 1998), showed that topically and orally administered Aloe vera preparations to patients with chronic venous leg ulcers aid healing. It has also been reported that many diabetic subjects take the Aloe vera gel because of its hypoglycemic properties (Okyar et al. 2001). However, it does not only possess hypoglycemic properties but also has hypotensive, hepatoprotective and blood purifying properties (Tiwari and Rao, 2002). Therefore, the present investigation was carried out to study the effect of low dose of fresh Aloe vera leaf gel extracts on in vitro sperm parameters for asthenozoospermic patients.

Materials and Methods:

- Patients and semen analyses:

Thirty infertile patients with asthenozoospermia were involved in this study during their attendance to High Institute for Infertility Diagnosis and Assisted Reproductive Technologies /Al-Nahrain University. Semen was collected after 3 to5 days of abstinence directly into clean, dry and sterile disposables Petri-dishes by masturbation in a room near the laboratory. The specimens were placed in incubator at 37°C for 30 minutes to allow liquefaction, and then these specimens were examined in details by macroscopic and microscopic examinations with vitality test. The preparations were scored under magnification of 40X objectives. The motility of each spermatozoon was graded as A- rapid progressive motility, B-slow progressive motility, C- non- progressive motility and D- immotile. A drop of semen was placed in 1% eosin and 5% nigrosin in 3% sodium citrate dehydrates solution for the live/dead ratio and Hypo- osmotic swelling Test (HOST) was also done (Lui, et al. 1997).Sperm parameters were assessed pre- and post treatment with 5 µL of Aloe vera gel extract.

- Aloe vera extract:

Fresh leaves of *Aloe vera* having a length of approximately 15 to 25 cm were washed with fresh water. The leaves were cut transversely into pieces. The solid gel in the center of the leaf was homogenized then, the filtrate of *Aloe vera* extract was obtained manually with care to avoid contamination of the gel. The clear filtrate was kept at 20°C until used (Subbiah et al. 2005). Five µL of fresh *Aloe vera* leaf gel extract was mixed with 1 mL of liquefied semen and left in the incubator for 1 hour, and then sperm parameters including concentration, morphology, agglutination, round cells, motility, and vitality were evaluated in the samples. Data from treated and control groups are expressed as mean ± standard error (SEM) and analyzed using student t-test to compare values from experimental and control groups at individual time periods. Differences between groups were considered significant at ($P<0.05$) and highly significant at ($P<0.01$) (Daniel, 1988).

Results:

In the present study highly significant increment ($P\leq 0.01$) was recorded in the sperm concentration and in the percentage of normal sperm morphology, while a highly significant decrease ($P\leq 0.01$) was observed in the percentage of agglutination and round cells number in comparison with pre-treated group as shown in table (1).As compared to pre-treatment, highly significant increase at ($P\leq 0.01$) was assessed in the percentage of sperm motility, progressive motility, grade A, and highly significant decrease ($P\leq 0.01$) was found in the grade D. While, a significant difference ($P\leq 0.05$) was recorded in percentage of grade C and non significant difference ($P\leq 0.05$) was recorded in grade B (%) in comparison with pre-treated group as shown in table (2).The results of this study revealed a highly significant increase ($P\leq 0.01$) was showed in the percentages of sperm viability and HOST post-treatment when compared with pre-treated group as shown in table (3).

Discussion:

The results of this study showed that there is highly significant increase in the sperm concentration, this may be due to the role of antioxidative phenolic compound in *Aloe vera* and its effects on lipid peroxidation (Yuji Ikeno et al. 2002). Phenolic compound have been postulated to be enhancers by altering arachidonic acid metabolism, stabilizing the lysosomal membrane, and protecting nuclear structure (Prabhala et al. 1990). Phenolic antioxidants have also been found to function as free radical terminators or metal chelators (Shahidi et al. 1992). The result of the present study also revealed that there is highly significant increase ($P\leq 0.01$) in the normal sperm morphology, motility, grade A and significant decrease at ($P\leq 0.05$) in grade C. These results can be explained by that spermatozoa are also responsible for production of reactive oxygen species (ROS) (Aitken, 1995, Whittington and Ford, 1999). which negatively affect the sperm plasma membrane by causing phospholipids peroxidation, hence decreased membrane fluidity and impaired sperm function, and also affect the sperm DNA by causing strand breaks that can be revealed by various tests of sperm DNA integrity such as nick translation (Sakkas et al. 1997) and the sperm chromatin structure assay (SCSA) (Evenson, 1999). The deleterious effect of free radicals or so called ROS upon sperm function and the hazardous effects of ROS during preparation *in vitro* was described previously (Aitken et al. 1989). Furthermore, ROS formation in an *in-vitro* condition has been associated with decreased sperm motility, abnormal morphology and a lowered capacity for sperm-oocyte penetration (Aitken and Fisher, 1994). However, only those spermatozoa with excess retained spermatid cytoplasm generate ROS (Huszar et al. 1999). So, several workers have suggested including antioxidant protection in sperm preparation media formulation (David, 2000). Despite the extensive history and popular acceptance of products containing *Aloe vera*, only a few articles have discussed *Aloe* antioxidants and their physiological effects in biological systems (Grindlay and Reynolds, 1986). This antioxidant activity can explain the results of the present study which showed highly significant increase in the percentage of sperm motility and normal sperm morphology. This result agreed with a recent work of Jasem and Nasim, 2011 who cited that *Aloe vera* may enhance male fertility by elevating sperm quality and has spermatogenic activity due to chemical compounds in it and may be useful to produce drugs for improve male fertility (Krausz et al. 1992). A significant decrease in the sperm viability and HOS test for negative semen samples of pre-treated group

may attribute to the biological and or physiological roles of oxidative processes and toxic free radicals (Halliwell,1987). Evidence has accumulated supporting the role of ROS in the pathogenesis of sperm dysfunction among men with infertility (Aitken and Clarkson, 1987). Furthermore, inflammation and immunologic reaction against the sperm and male genital tract cause leukocytospermia that affect sperm cells, directly or indirectly, through enhanced phagocytosis and result in defective sperm parameters and function (Buch et al.1994, Rajasekaran et al.1995). Semen samples with increased numbers of leukocytes have been shown to have high levels of ROS (Wolff et al. 1990, Naz et al.1994). The established use of Aloe preparations in traditional medicine for treatment of a variety of disorders might be attributed, to some degree, to the antioxidant activity of its components (Ki Young et al. 2000). This indicates that the continuous administration of Aloe vera extract give a highly significant increase in sperm viability and HOS test demonstrated. Decrease number of round cells and agglutination may be due to the anti- inflammatory activities of components from Aloe species (Davis et al. 1989) and the antibiotic properties of this plant extract (Hu et al. 2003). This result agreed with the study of Davis and his co-workers (Davis et al. 1991), who proposed that Aloe gel contained both an inhibitory system and a stimulatory system that influenced both inflammatory and immune responses. Ahmad and his co-workers (Ahmad et al. 1993) and Hutter and his co-workers, 1996 also often mentioned the antibacterial, antifungal and even antiviral properties demonstrated by the Aloe gel. It has also been demonstrated that they are able to modify the release of cytokine-like products after stimulation of lymphocytes and monocytes (Abril et al 1989), and thus may enhance immuno-surveillance through activation of natural killer cells (Prabhala et al. 1990). So, several studies have been conducted to identify natural phenolics that possess antioxidant activity; some of these substances are now being extracted from plant sources for commercial production (Schuler1990).

Conclusion

The using low dose of Aloe vera extract can enhanced sperm parameters especially in relation to motility, morphology and viability

Table (1): Effect of Aloe vera extracts on sperm parameters for infertile patients with asthenozoospermia

Sperm parameters	Sperm concentration (million/ml)	Normal sperm morphology (%)	sperm Agglutination (%)	Round cells
Pre-treatment	73.93 ± 17.33	13.39 ± 1.14	12.7 ± 2.56	8.02 ± 1.07
Post-treatment	94.73** ± 7.23	27.12** ± 1.97	2.72** ± 0.63	3.8** ± 0.57

**Highly significant (P≤ 0.01) difference.No. of semen samples: 30.

Table (2): Effect of Aloe vera extracts on sperm motility and grade activity for infertile patients with asthenozoospermia

Sperm parameters	Sperm motility (%)	Progressive motility (%)	Grade A (%)	Grade B (%)	Grade C (%)	Grade D (%)
Pre Treatment	61.88 ± 4.17	37.39 ± 3.1	6.06 ± 1.98	31.33 ± 2.8	24.49 ± 3.67	38.12 ± 3.42
Post Treatment	74.46** ± 4.6	55.10** ± 3.62	22.34** ± 4.22	32.76 ± 3.91	19.36* ± 2.69	25.54** ± 3.58

*Significant (P≤ 0.05) difference.**Highly significant (P≤ 0.01) difference.No. of semen samples: 30.

Table (3): Effect of Aloe Vera extracts on sperm viability and HOST for infertile patients with asthenozoospermia

Sperm parameters	Sperm viability (%)	HOS test (%)
Pre-treatment	66.31 ± 2.9	64.24 ± 2.35
Post treatment	84.46** ± 3.25	79.54** ± 3.02

**Highly significant (P≤ 0.01) difference.

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