

Review article

Antioxidants and infertility treatment, the role of *Satureja Khuzestanica*: A mini-systematic review

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Abstract

Background: The use of antioxidants in the treatment of infertility has been suggested and recent studies have indicated that oral administration of *Satureja Khuzestanica* essential oil (SKEO) to rats induces significant antioxidative effects.

Objective: This systematic review was conducted to evaluate the effectiveness of antioxidants in infertility and also to assess the effectiveness of *Satureja Khuzestanica* in infertility management.

Materials and Methods: Pubmed, Scopus, and Cochrane Library were searched for relevant trials published from respective database inception dates to August 2010. Study selection, and data extraction were performed by authors.

Results: Fifteen trials on evaluation of antioxidants in infertility and seven studies on the effectiveness of *Satureja Khuzestanica* were identified. Only 4 of the 15 trials were with application of allocation concealment and three studies were done in in-vitro environment. Despite the methodological and clinical heterogeneity of the trials, 14 of the 15 (93.33%) trials showed an improvement in either sperm quality or pregnancy rate after antioxidant therapy. The human and animal studies of *Satureja Khuzestanica* showed a significant antioxidative potential of the plant and its effectiveness for infertility improvement.

Conclusion: The use of oral antioxidants in infertility could improve sperm quality and pregnancy rates. Improved fertility observed by SKEO in rats might be due to its antioxidative effect. Further studies and clinical trials in humans are necessary to evaluate SKEO effectiveness in fertility disturbances.

Key words: Antioxidant, Infertility, *Satureja Khuzestanica*.

Introduction

According to several studies, presence of oxidative stress could cause molecular and genetic defects leading to infertility (1). Oxidative stress is usually associated with aerobic metabolism that generate pro-oxidant molecules (free radicals) or reactive oxygen species (ROS) (including hydroxyl radicals, superoxide anion, hydrogen peroxide, and

nitric oxide). There are complex interactions between the prooxidants and antioxidant molecules resulting in maintenance of intracellular homeostasis. Whenever there is an imbalance between the pro-oxidants and antioxidants, a state of oxidative stress is initiated. Some cells possess specific mechanisms to produce ROS required for cellular functions in low concentrations. Depending on ROS tissue concentration, they can exert beneficial physiologic effects and play a role in fertilization processes. For example, free radicals can influence the oocytes, sperm, and embryos in their micro environments, including follicular fluid, hydrosalpingeal fluid, and peritoneal fluid. These micro environments have a direct effect on quality

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of oocytes, sperm oocyte interaction, implantation, and early embryo development. Free radicals can also have pathological damage to cellular components including lipids, proteins and nucleic acids. There is a complex interplay of cytokines, hormones, and other stressors (such as irradiation, e.g. UV sunlight) that affects cellular generation of free radicals; these molecules act further through the modification of many transcription factors and gene expression (2). The modification may result in structural and functional alterations and impair many cellular processes. Pathological mechanisms of cell injury include lipid peroxidation, DNA damage, and apoptosis. Organisms have developed efficient protective mechanisms against excessive accumulation of ROS. ROS could be neutralized by an elaborate antioxidant defense system consisting of enzymes such as catalase, superoxide dismutase and glutathione peroxidase/reductase, and numerous non-enzymatic antioxidants such as vitamin C, vitamin E, vitamin A, pyruvate, glutathione, taurine and hypotaurine.

Whenever ROS levels become pathologically elevated, antioxidants begin to work and help to minimize the oxidative damage, repair it or prevent it altogether. The male and female genital tracts are rich in both enzymatic and non-enzymatic antioxidants. Follicular fluid contains high levels of antioxidants, which protect oocytes from ROS-induced damage. Significantly lower selenium levels were detected in follicular fluid of patients with unexplained infertility compared with those with tubal infertility or couples with male factor infertility. Elevated levels of ROS in peritoneal fluid may be the cause of infertility in some women with no other obvious causes.

Elevated levels of ROS can damage the ovum (after its release from the ovary), the zygote/embryo and most importantly, spermatozoa. Spermatozoa are very sensitive to oxidative stress. Based on some studies, 30-80% of unselected infertile men have oxidative stress-related infertility. Oxidative stress appears to be due to increased generation of ROS rather than a depletion of antioxidants. Therefore it is important to identify the source of increased ROS generation.

Antioxidant supplementation may or may not be effective depending on the pathology of the infertility (3). A number of drugs with antioxidative properties have been postulated to have a possible role in the management of idiopathic problems. However, WHO recommends application of traditional drugs in medical health service system. So recently, there has been a significant interest in finding natural antioxidants from plant materials to replace synthetic medicines (4). New studies have

indicated that oral administration of *Satureja Khuzestanica* essential oil (SKEO) to rats induces a significant antioxidative property without appearance of any toxicity or unwanted effects.

Satureja Khuzestanica is an endemic plant in the Southern part of Iran. Its fame is due to the medical uses as analgesic and antiseptic in folk medicine resulted from the essential oil existing in that. There are marked differences between and within the subspecies of *Satureja* essential oil composition in the world. Recent studies indicated significant antioxidative, antidiabetic, antihyperlipidemic, and reproduction stimulatory effects for oral administration of SKEO to rats.

No toxicity or adverse effects were observed (5) and also a significant increase in the number of implantation and live fetuses in female rats receiving SKEO was investigated (4). The present work aimed to review the effect of antioxidative drugs in infertility and the effectiveness of *Satureja Khuzestanica* in treating the problem.

Materials and methods

To investigate the effect of antioxidants on infertility, published literature was obtained by searching MEDLINE on the Pubmed search system and databases on Scopus interface. Filters were applied to limit the retrieval to randomized controlled trials. Regular alerts were established on MEDLINE and information retrieved through alerts is current to August 2010.

Parallel searches were performed on Cochrane library with all record status and No filters were applied to limit the retrieval by study type. The key words, antioxidants and infertility were used and controlled vocabulary such as the National Library of Medicine's MeSH (Medical Subject Headings) and keywords were applied. The search was restricted to English language articles on human population. The articles obtained were screened based on the title and abstract and then selected for inclusion in the report. The criteria for article inclusion were study design (mainly randomized controlled trials), population (patient of any age with any primary problem of infertility were included in the study), intervention (any antioxidant), comparator (mainly placebo) and outcome (any clinical outcome). Duplicate publications of identical trials were excluded. Full text articles were obtained for all selected articles if possible and abstract was used for the rest. The Cochrane Reviewers' Handbook applied to evaluate the quality of the articles. To investigate the effectiveness of *Satureja Khuzestanica*, the key word field of Pubmed and Scopus were searched

without any limitation to be implicated. The search term was “*Satureja Khuzestanica*” and all the relevant literature was included in the study.

Results

By initial searching, a collection of 83 articles were obtained of which 22 studies (5-26) were identified based on their titles and abstracts and all of them included in the review. 15/ 22 of the studies were about the effectiveness of antioxidants in infertility (6-20) (Table I) and 7/ 22 of them were concerning the effectiveness of SKEO (5, 21-26) (Table II). Only 4 of the studies were randomized controlled trials with allocation concealment (6-9) and 3 of them were performed in in Vitro environment (13, 15, 20).

Based on the study by Keskes-Ammar *et al* sperm motility and viability are inversely correlated with semen MDA (Malonaldehyde is produced from oxidation of polyunsaturated fatty acids) levels (12). Spermatozoal MDA concentration is also higher in men with decrease sperm motility (19). Also DNA methylation has a significant negative correlation with sperm DNA fragmentation and seminal reactive oxygen species (ROS) production (16). The first observation of a correlation between the anti-oxidant substances and ROS generation in human semen was reported in 1995 (18) which are supported by several of other studies (11, 12, 16, 19). According to references (6, 8, 11, 12) (Table I), antioxidative supplementation produces a significant improvement of sperm motility. Two of the included studies showed that the total and per cycle pregnancy rates among the cases treated with antioxidant is higher than the control group (11, 19). The percentage of DNA-fragmented spermatozoa is markedly reduced by antioxidative drug therapy (7, 14, 15, 20). Antioxidative therapy may also be effective on improving the sperm count at least in a subset of oligospermic males (9). Proven fertile men has higher blood and spermatozoa levels of omega-3 fatty acids compared with the infertile patients and the ratio of serum omega-6/omega-3 fatty acids is significantly higher in infertile patients in comparison to fertile controls (17). Among all the evidences supporting the usefulness of anti oxidants in infertility, in the study by Tarin *et al* no significant effect of Ascorbate on fertilization, number of cells and embryo grade or percentage of embryos was observed (13). The information obtained from the studies evaluating the effectiveness of SKEO, are summarized in table II.

In the study of Vosough-ghanbari *et al* in 2008, the patients treated with SK showed significant

decrease in total cholesterol, LDLC and increase in HDL-C and total antioxidant power (TAP) compared to baseline while the patients in the placebo group showed no changes (21). In the study by Abdollahi *et al* (22), SKEO therapy decreased the normal lipid peroxidation level and increased significantly the antioxidant capacity. Blood glucose and triglycerides levels were also decreased significantly. In the study by Ghazanfari *et al* (23), elevated lipid peroxidation and myeloperoxidase activity was significantly restored by SKEO administration, also SKEO-treated groups showed significantly lower score values of macroscopic and microscopic characters when compared to the experimentally induced IBD group. Generally, the beneficial effect of SKEO was comparable to that of prednisolone. Saadat and colleagues (24) showed that SKEO therapy did not affect on the blood glucose levels but hepatic phosphoenolpyruvate carboxykinase (PEPCK) activity decreased by 26% and hepatic glycogen phosphorylase (GP) increased by 24% in comparison to control group. One of the studies included in our review showed that SKEO could protect rats treated with cyclophosphamide from hemorrhagic cystitis (25) and in another study; protection of the reproductive system in these animals was observed (26).

The effects of SKEO on infertility were investigated by Haery *et al* (5) in which the potency and fecundity of the male rats administered SKEO were significantly higher than those of the age-matched control group. Fertility index and litter size were also significantly improved as well as decreasing in post implantation loss in mated females of the treated groups. There was no effect on the serum LH by SKEO therapy but concentration of FSH increased significantly in the group treated with SKEO. SKEO increased significantly serum testosterone levels in all doses used but no change was observed in serum concentration of estradiol. In male rats treated with SKEO, the number of spermatogonium, spermatid cords, Leydig cells, and spermatozooids was increased according to histopathological analysis. The sertoli cells were also hypertrophic in this group.

In our searching strategy, no appropriate RCT on infertility in women was found, however we encountered with the study of Ghanem *et al* in which significantly higher pregnancy rate was found among the patients treated with combination of clomiphene and an antioxidative drug (10). Also Tamura and colleagues investigated the effect of melatonin, an antioxidant, on intra-follicular oxidative stress in vitro and realized their significant negative relationship (20).

Table I. Human studies evaluating antioxidants effectiveness in infertility.

Study	Objective	Type	Target/ Age (year)	Setting	Case Control No.	Regimen	Outcome
Vicari et al. 2002 (6) (Italy)	Whether removal of prooxidant factors may make administration of carnitin more beneficial in patients with PVE	Open, prospective, random study	98 men with PVE who had increased seminal leukocyte concentrations ($>1 \times 10^6$ cells/mL) / 22-42 years	Academic research environment	30/16/26/26	Cantinites, 4m / NSAIDs, 4m/ NSAIDs, 2m then Carnitines, 2m/ NSAIDs + carnitines, 4m	Semen variables, production of reactive oxygen species, and pregnancy outcome were evaluated before and after treatment and following a 3-month washout period.
Tremellen et al. 2007 (7) (Australia)	To examine the effect of male antioxidant treatment on embryo quality and pregnancy outcome during IVF-ICSI treatment	Prospective randomized double-blind placebo-controlled trial	60 couples undergoing IVF-ICSI/ Male: 37.1 ± 5.1 years Female: 34.6 ± 3.4 year	A single ART unit	Ratio: 2/1	Either one capsule per day of the Menevit antioxidant or an identical placebo for three months prior to their partner's IVF cycle	Primary outcome was number of good quality embryos generated per IVF cycle. Pregnancy, utcome, fertilisation rates and male side-effect profiles were secondary outcomes.
Rolf et al. 1999 (8) (Germany)	To clarify whether supplementation with vitamins C and E can protect human semen from possible damage by oxygen free radical species in the epididymis, and thus improve semen parameters	Single-centre, double-blind, placebo-controlled, truly randomized study	31 men with infertility persisting longer than one year/ 35.2 ± 4.8 years	Institute of Reprod. Med. of the University	15/16	Patients were given either 1000 mg vitamin C and 800 mg vitamin E daily, or placebo capsules.	Ejaculate analysis (included physical parameters (ejaculate volume, pH, colour) as well as spermatozoa concentration, motility and morphology. Also Sperm survival was examined by monitoring the percentage of viable cells before and after storage.
Paradiso Galatioto et al. 2008 (9) (Italy)	To deter the effect of an antioxi. therapy to improve the quality of seminal fluid parameters and the natural preg	Randomized, prospective, controlled, intention to treat study	42 men who all had performed a retrograde embolization with concomitant oligospermia/ 23-36 years	-		(NAC 600 mg and vitamins-minerals) for 90 days / Untreated group (22 subjects) received no adjunctive medical therapy was used as controls	Sperm count (millions/ml), percentage of WHO class A motile sperm and percentage of typical forms. As secondary endpoint the natural pregnancies were assessed
Ghanem et al. 2010 (10) (Egypt)	To assess the effect of treatment with a combination of clomiphene citrate and vitamin E on the incidence of pregnancy and sperm variables	Prospective, randomized, placebo-controlled trial	Sixty infertile men with idiopathic oligospermia/oligospermia/ 20-40 years	The outpatient andrology clinic at a university hospital	30/30	Clomiphene citrate (25 mg/day) + vitamin E (400 mg) once daily / placebo group maintained for 6 months.	Pregnancy incidence and variations in semen parameters.
Comhaire et al. 2005(11) (Belgium)	To assess the effects of complementary treatment with a strong lipophilic antioxidant on sperm function and fertility of subfertile men	Double blind, randomized trial design	30 men with infertility of ≥ 12 months/ 33.2 ± 5.6 years		11/19	Astaxanthin 16 mg daily / placebo for 3 months.	The effects of treatment on semen parameters, ROS, zona-free hamster oocyte test, serum hormones including testosterone, LH, FSH and Inhibin B, and spontaneous or IUI-induced pregnancies
Keskes-Ammar et al. 2003 (12) (Tunisie)	To evaluate the stress oxidative status in the sperm and the effects of an oral vitamin E and selenium on lipid peroxidation and on semen quality, in comp. with vitamin B treatment	Randomized and open	78 men with mean length of infertility 62 months (range 6-156)/ $23-67$ (35.5 ± 6.8) years		28 / 26 / 20	Vitamin E (400 mg) and selenium (225 mg), during 3 months / vitamin B (4.5 g daily) for the same duration ment and returned for control analysis	MDA concentrations in sperm and seminal plasma and the motility and viability of the sperms
Tarin et al. 1994 (13) (Spain)	To evaluate whether ascorbate, can improve fertilization and development of human embryos in vitro	Randomized and open	Human oocytes, spermatozoa and embryos from 83 infertile IVF couples	In vitro	39 / 44	Presence of ascorbate in HTF medium / absence of ascorbate	Fertilization and embryo development

Table I Contd. Human studies evaluating antioxidants effectiveness in infertility.

Study	Objective	Type	Target/ Age (years)	Setting	Case Control No.	Regimen	Outcome
Greco E 2005 (14)	To find out if the pathologically increased incidence of DNA fragmentation in ejaculated spermatozoa can be reduced by oral treatment with two antioxidants, vitamins C and E.	Prospective, placebo-controlled and double-blinded analysis	Sixty-four men with unexplained infertility and an elevated percentage of DNA-fragmented spermatozoa in the ejaculate		30/30	Antioxidant treatment (1 g vitamin C + 1 g vitamin E daily for 2 months) group / placebo group.	Sperm DNA fragmentation
Sierens J 2002 (15) (United Kingdom)	To test the antioxidant effects of the isoflavones, genistein and equol on sperm DNA integrity, assessed in vitro		Human sperm was collected from a healthy male donor (smoker, aged 27) after three days sexual abstinence	In Vitro			Baseline sperm DNA integrity
Tunc 2009 (16) (Australia)	To investigate the possible link between seminal oxidative stress related DNA fragmentation, serum homocysteine and methylation of sperm DNA.		Men with known male factor infertility semen parameters.	An academic affiliated ART unit	32/ 12	One capsule of Menevit® per day for a period of 3 months and	Assessment of sperm amethylation and DNA fragmentation, ROS production, Homocysteine analysis, Identification of leukocytes in semen CD45 measurement
Safarnejad M 2010 (17) (Iran)	To evaluate PUFA composition of the blood plasma and spermatozoa in infertile men with idiopathic OAT.		Men with idiopathic OAT and seventy-eight fertile men defined according to semen concentration and proven fertility were enrolled in the Eighty-two infertile study.	Infertility clinic	82 / 78		The semen parameters were assessed according to World Health Organization criteria
Thiele JJ 1995 (18) (Germany)	To investigate the relationship between the oxidative and anti-oxidative potential in semen of infertile patients and healthy donors		Patients who were classified as asthenospermic	Asir Infertility Center (AIC)	52 / 35	100 mg vitamin E or a placebo three times a day for six months	MDA concentration in spermatozoa and improved sperm motility
Suleiman SA 1996 (19) (Saudi Arabia)	To identify the major abnormality in semen parameters, level of lipid peroxidation and compare the effects of vitamin E and placebo	Random double-blind fashion	Eighteen patients undergoing IVF-ET who failed to become pregnant in the previous IVF-ET cycle were enrolled/ 24-45 year	In Vitro	56 / 59	3 mg tablet of melatonin orally at 22:00 hr from the fifth day of the previous menstrual cycle until the day of oocyte retrieval	Fertilization rate

PVE: Prostatovesiculopididymitis, NSAIDs : Non Steroidal Antiinflammatory Drugs , IVF-ICSI: In Vitro Fertilization-Intracytoplasmic Sperm injection, ART, ROS: Reactive oxygen Species, LH: Luteinizing Hormone, FSH: Follicle Stimulating Hormone, IUI: Intrauterine insemination, PUFA: poly Unsaturated Fatty Acid, OAT: Oligoasthenozoospermia, IVF-ET: In Vitro Fertilization and Embryo Transfer

Table II. The studies evaluating the anti oxidative effects of *Satureja Khuzestanica* essential oil.

Human Study							
Study	Objective	Type	Target (age/year)	Setting	Number (Case/Control)	Regimen	Outcome
Vosough-Chanbar et al. 2008 (21) (Iran)	To investigate the antidiabetic, antihyperlipidemic and antioxidant effects of <i>S. Khuzestanica</i>	Randomized in a double blind, placebo controlled clinical trial	Twenty-one (12 male and 9 female) hyperlipidemic patients with type 2 diabetes mellitus	Outpatient clinic of a University Hospital	11/12	<i>S. khuzestanica</i> a tablets (250 mg) once a day, 2 m / placebo with the same regimen.	Significant decrease in total cholesterol, LDLC, and increase in HDL-C, and TAP measured from baseline to 2 months in the <i>satureja khuzestanica</i> group.
Animal Studies							
Study	Objective	Target	Dose/day	Route of administration	Duration of treatment	Other groups	Effect
Abdollahi et al. 2003 (22)	To investigate the antidiabetic, antihyperlipidemic and anti-oxidative stress effects of (SKEO) on rat blood in vivo.	Diabetic and hyperlipidemic rats	1000 ppm	Oral (drinking water)	10 d	Control	LPO decreased and TAC increased
Ghazanfari et al. 2006 (23)	To evaluate its activity against inflammatory bowel disease	Mouse model of IBD	500, 1000, 1500 ppm	Oral	7 d before and 10 d after Induction of IBD	Prednisolone	MPO and LPO increased in IBD and significantly restored by herbal essence (1500 ppm) that was comparable to effect of prednisolone
Saadat et al. 2004 (24)	To examine whether administration of SKEO might affect activities of hepatic key enzymes of gluconeogenesis and glycogenolysis including (PEPCK) and (GP) in rat.	Healthy rat	1000 ppm	Oral (drinking water)	2 w	Control	Hepatic PEPCK decreased and GP increased
Haeri et al. 2006 (5)	To ascertain the effect of SKEO on the reproductive behavior and fertility of male rats during prolonged oral treatment.	Healthy male rat	75, 150, and 225, mg/kg/day	Oral (drinking water)	45 d	Control	Significant improvements in potency, fecundity, fertility index, and litter size and significant decrease in post implantation loss
Rezvanfar 2009(25)	To investigate protective effects of SKEO in a subchronic rat model of CP-induced HC	Rats with CP-induced HC	CP (6 mg/kg) SKEO (225 mg/kg)	Oral (distilled water)	28 d	SKEO alone or SKEO + CP	Protection rats from CP-induced HC by reduction of free radical-induced toxic stress
Rezvanfar et al. 2008 (26)	To examine whether coadministration of SKEO can prevent CP-induced reproductive toxicity in rats and how free radicals play a role.	Male rat treated with CP	CP (6mg/kg/day), SKEO (225 mg/kg/day)	Oral	4 w	Olive oil, SKEO, SKEO + CP	SKEO protected reproductive system from toxicity of CP through its antioxidant potential and androgenic activity.

TAP: Total Antioxidant Power, SKEO *Satureja Khuzestanica* essential oil, LPO: Lipid Per-oxidation, TAC: Total Antioxidant Capacity, W: Week PEPECK: Phosphoenolpyruvate carboxykinase, GP: glycogen Phosphorylase, HC: Hemorrhagic Cystitis , CP: Cyclophosphamide

Discussion

The results obtained from the trials included in our mini-review, confirm the protective and usefulness of antioxidants on infertility and reproduction system. For example, the higher blood and spermatozoa levels of omega-3 fatty acids in comparison to the infertile patients (17), is in agreement with the usefulness of antioxidants in infertility because Omega-3 fatty acids are known potentially important antioxidants. These substances are an element in all cell membranes and maintain the properties of the lipid bilayers. The lipid bilayers also exist in the spermatozoa membrane and are essential for the fluidity and flexibility of spermatozoa, and successful fertilization.

There is also a close relationship between oxidative stress and poor oocyte quality (20) but unfortunately in our searching strategy, no randomized clinical trial was found on women infertility treatment with antioxidants. Therefore appropriate controlled studies designing in this population is needed.

However, Ghanem *et al* observed significantly higher pregnancy rate among the patients treated with combination of clomiphen and an anti-oxidative drug (10). But there was no comparison between a group treated with antioxidant alone and a group as control. Therefore the effectiveness of the antioxidant used in the trial, could not be judged well.

Despite all the evidences represent the usefulness of anti oxidants in infertility, the study of Tarin *et al* showed no significant effectiveness of ascorbate on fertilization, number of cells and embryo grade or percentage of embryos. However, the positive effect of antioxidants on fertilization and embryo development in vitro cannot be totally ruled out until the effects of other, non-physiological concentrations of ascorbate and longer-term embryo cultures have been tested (13).

Given the effectiveness of antioxidants in management of infertility, *Satureja Khuzestanica* which has established antioxidative properties (22, 23) could be proposed to represent reproduction stimulatory effects.

Of the studies in which the antioxidative moiety of SKEO has been assessed, the trial of Vosoughghanbari *et al* could be pointed which was performed in 2008 and showed the patients treated with SK, represent significant decrease in total cholesterol, LDLC and increase in HDL-C and total antioxidant power (TAP) compared to baseline (21). Generally, it appears that plants particularly those with high levels and strong antioxidative compounds have an important role in improvement of disorders relating to oxidative stress such as diabetes mellitus (27).

SKEO which has antioxidative properties should be useful in diabetes and its complications. The results obtained from the study also confirmed the effectiveness of SK on improving patients' total cholesterol, LDL-C, HDL-C and TAP (21).

Ghazanfari *et al* (23) showed significantly lower score values of macroscopic and microscopic characters were observed, compared to the experimentally induced IBD group. As elevated lipid peroxidation and myeloperoxidase activity was also significantly restored by SKEO administration, it seems that antioxidative property is one of the mechanisms by which this plant might have protective effects.

Saadat *et al* (24) realized that SKEO therapy did not affect the blood glucose levels but decreased hepatic *phosphoenolpyruvate carboxykinase* (PEPCK) activity by 26% and increased hepatic glycogen phosphorylase (GP) by 24% in comparison to control group. Disturbance of hepatic glucose metabolism has been proposed as a mechanism of anti-diabetic action of SKEO which could be in relation with antioxidative effect of this plant.

The PEPCK gene in liver is present in most models of diabetes, and is thought to contribute to the increased hepatic glucose output seen in diabetes. Thus any medicine to alter hepatic gluconeogenesis or glycogenolysis might have significant effect on glucose hemostasis.

According to existing reports, troglitazone also inhibits expression of the PEPCK gene in isolated hepatocytes by an antioxidative property which is due to continuation of the alpha-tocopherol (vitamin

E) moiety in its chemical structure (28). Furthermore metformin which inhibits hepatic gluconeogenesis, produces concurrent antioxidative effects and have most benefits in treatment of diabetes (29) in view of the ability of SKEO to reduce hepatic PEPCK activity and considering above statements, the first mechanism that may come to mind is its antioxidative properties.

The human and animal studies of *Satureja Khuzestanica* show significant antioxidative potential of the plant. Injury of the plant cells is associated with the occurrence of oxidative mechanisms that may explain why an abundance of antioxidative compounds have been extracted from plant tissue (4). Various animal models including inflammatory bowel disease, diabetes, and hyperlipidemia in rat have been investigated and most of them have been treated by SKEO according to its antioxidative properties. As a result, the effectiveness of SKEO in infertility could be assumed by considering its antioxidative properties. Of course this hypothesis has been established in rats by the study of Haeri *et al* in which histopathological analysis in male rats treated with SKEO demonstrated the increase of the number of spermatogonium, spermatid cords, Leydig cells, and spermatozooids and hypertrophic Sertoli cells (5). The result of the study also supports the previous discovery on the reproduction stimulation indicated by enhancing the number of live fetuses per litter in dams treated by SKEO (22). The results were approved by decreased blood lipid peroxidation and increased total antioxidant power (5).

Flavonoids, mainly p-cymene and carvacrol have been documented in the SKEO and have been well analyzed by GC-mass. Their positive effects are due to their ability to inhibit lipid peroxidation, chelate redox-active metals, and attenuating other processes involving reactive oxygen species. Also carvacrol represents significant antioxidant properties (30-32). The increase in the weight of the testis and weights of the epididymis, seminal vesicles and ventral prostate in SKEO-treated rats would be the result of decrease in lipid peroxidation. Therefore, improved fertility observed in the study of Haeri *et al* might be due to the antioxidant effect of SKEO.

Conclusion

Although the methods and clinical settings of the controlled trials included in our mini-systematic review were diverse, 14/ 15 (93.33%) of the trials proved that there is an improvement in either sperm quality or pregnancy rate after antioxidative therapy, however, randomized placebo clinical trials with adequate power are needed to guide clinical practice (33).

As the antioxidative properties of SKEO have been established and its effectiveness also has been observed in vitro, its effectiveness in human infertility could be hypothesized. Further preclinical evaluations and clinical trials in humans are necessary to identify a possible place for SKEO in therapies of infertility (5).

References

1. Agarwal A, Prabakaran S, Allamaneni SS. Relationship between oxidative stress, varicocele and infertility: a meta-analysis. *Reprod Biomed Online* 2006; 12: 630-633.
2. Gupta S, Agarwal A, Banerjee J, Alvarez JG. The role of oxidative stress in spontaneous abortion and recurrent pregnancy loss: a systematic review. *Obstet Gynecol Surv* 2007; 62: 335-347.
3. Agarwal A, Allamaneni SSR. Oxidants and antioxidants in human fertility. *Middle East Fertility Society Journal* 2004; 9: 187-197.
4. Hasani-Ranjbar S, Larijani B, Abdollahi M. A systematic review of the potential herbal sources of future drugs effective in oxidant-related diseases. *Inflamm Allergy Drug Targets* 2009; 8: 2-10.
5. Haeri S, Minaie B, Amin G, Nikfar S, Khorasani R, Esmaily H, et al. Effect of *Satureja khuzestanica* essential oil on male rat fertility. *Fitoterapia* 2006; 77: 495-499.
6. Vicari E, Vignera S La, Calogero AE. Antioxidant treatment with carnitines is effective in infertile patients with prostatovesiculopididymitis and elevated seminal leukocyte concentrations after treatment with nonsteroidal anti-inflammatory compounds. *Fertil Steril* 2002; 78: 1203-1208.
7. Tremellen K, Miari G, Froiland D, Thimpson J. A randomised control trial examining the effect of an antioxidant (Menevit) on pregnancy outcome during IVF-ICSI treatment. *Aust N Z J Obstet Gynaecol* 2007; 47: 216-221.

8. Rolf C, Cooper TG, Yeung CH, Nieschlag E. Antioxidant treatment of patients with asthenozoospermia or moderate oligoasthenozoospermia with high-dose vitamin C and vitamin E: a randomized, placebo-controlled, double-blind study. *Hum Reprod* 1999; 14: 1028-1033.
9. Paradiso Galatioto G, Gravina GL, Angelozzi G, Sacchetti A, Innominato PF, Pace G, et al. May antioxidant therapy improve sperm parameters of men with persistent oligospermia after retrograde embolization for varicocele? *World J Urol* 2008; 26: 97-102.
10. Ghanem H, Shaeer O, El-Segini A. Combination clomiphene citrate and antioxidant therapy for idiopathic male infertility: a randomized controlled trial. *Fertil Steril* 2010; 93: 2232-2235.
11. Comhaire FH, El Gareem Y, Mahmoud A, Eertmans F, Schoonjans F. Combined conventional/ antioxidant" Astaxanthin" treatment for male infertility: a double blind, randomized trial. *Asian J Androl* 2005; 7: 257-262.
12. Keskes-Ammar L, Feki-Chakroun N, Rebai T, Sahnoun Z, Ghazzi H, Hammami S, et al. Sperm oxidative stress and the effect of an oral vitamin E and selenium supplement on semen quality in infertile men. *Arch Androl* 2003; 49: 83-94.
13. Tarin JJ, de los Santos MJ, de Oliveira MN, Pellicer A, Bonilla-Musoles F. Ascorbate-supplemented media in short-term cultures of human embryos. *Hum Reprod* 1994; 9: 1717-1722.
14. Greco E, Iacobelli M, Rienzi L, Ubaldi F, Ferrero S, Tesarik J. Reduction of the incidence of sperm DNA fragmentation by oral antioxidant treatment. *J Androl* 2005; 26: 349-353.
15. Sierens J, Hartley JA, Campbell MJ, Leatham AJ, Woodside JV. In vitro isoflavone supplementation reduces hydrogen peroxide-induced DNA damage in sperm. *Teratog Carcinog Mutagen* 2002; 22: 227-234.
16. Tunc O, Tremellen K. Oxidative DNA damage impairs global sperm DNA methylation in infertile men. *J Assist Reprod Genet* 2009; 26: 537-544.
17. Safarinejad MR, Hosseini SY, Dadkhah F, Asgari MA. Relationship of omega-3 and omega-6 fatty acids with semen characteristics, and anti-oxidant status of seminal plasma: A comparison between fertile and infertile men. *Clin Nutr* 2010; 29: 100-105.
18. Thiele JJ, Friesleben HJ, Fuchs J, Ochsendorf FR. Ascorbic acid and urate in human seminal plasma: determination and interrelationships with chemiluminescence in washed semen. *Hum Reprod* 1995; 10: 110-115.
19. Suleiman SA, Ali ME, Zaki ZM, el-Malik EM, Nasr MA. Lipid peroxidation and human sperm motility: protective role of vitamin E. *J Androl* 1996; 17: 530-537.
20. Tamura H, Takasaki A, Miwa I, Taniguchi K, Maekawa R, Asada H, et al. Oxidative stress impairs oocyte quality and melatonin protects oocytes from free radical damage and improves fertilization rate. *J Pineal Res* 2008; 44: 280-287.
21. Vosough-Ghanbari S, Rahimi R, Kharabaf S, Zeinali S, Mohammadirad A, Amini S, et al. Effects of Satureja khuzestanica on serum glucose, lipids and markers of oxidative stress in patients with type 2 diabetes mellitus: A double-blind randomized controlled trial. *Evid Based Complement Alternat Med* 2010; 7: 465-470.
22. Abdollahi M, Salehnia A, Mortazavi SH, Ebrahimi M, Shafiee A, Fouladian F, et al. Antioxidant, antidiabetic, antihyperlipidemic, reproduction stimulatory properties and safety of essential oil of Satureja Khuzestanica in rat in vivo: a toxicopharmacological study. *Med Sci Monit* 2003; 9: 331-335.
23. Ghazanfari G, Minaie B, Yasa N, Nakhai LA, Mohammadirad A, Nikfar S, et al. Biochemical and histopathological evidences for beneficial effects of Satureja Khuzestanica Jamzad essential oil on the mouse model of inflammatory bowel diseases. *Toxicol Mech Methods* 2006; 16: 365-372.
24. Saadat M, Pournourmohammadi S, Donyavi M, Khorasani R, Amin G, Nazar Salehnia A, et al. Alteration of rat hepatic glycogen phosphorylase and phosphoenolpyruvate carboxykinase activities by Satureja khuzestanica Jamzad essential oil. *J Pharm Pharm Sci* 2004; 7: 310-314.
25. Rezvanfar MA, Farshid AA, Sadrkhanlou RA, Ahmadi A, Salehnia A, Abdollahi M. Benefit of Satureja khuzestanica in subchronically rat model of cyclophosphamide-induced hemorrhagic cystitis. *Exp Toxicol Pathol* 2010; 62: 323-330.
26. Rezvanfar MA, Sadrkhanlou R, Ahmadi A, Shojaei-Sadee H, Mohammadirad A, Salehnia A, et al. Protection of cyclophosphamide-induced toxicity in reproductive tract histology, sperm characteristics, and DNA damage by an herbal source; evidence for role of free-radical toxic stress. *Hum Exp Toxicol* 2008; 27: 901-910.
27. Rahimi R, Nikfar S, Larijani B, Abdollahi M. A review on the role of antioxidants in the management of diabetes and its complications. *Biomed Pharmacother* 2005; 59: 365-373.
28. Davies GF, Khandelwal RL, Wu L, Juurlink BH, Roesler WJ. Inhibition of phosphoenolpyruvate carboxykinase (PEPCK) gene expression by troglitazone: a peroxisome proliferator-activated receptor- (PPAR) -independent, antioxidant-related mechanism. *Biochem Pharmacol* 2001; 62: 1071-1079.
29. Cosić V, Antić S, Pesić M, Jovanović O, Kundalić S, Djordjević VB. Monotherapy with metformin: does it improve hypoxia in Type 2 diabetic patients? *Clin Chem Lab Med* 2001; 39: 818-821.
30. Burits M, Bucar F. Antioxidant activity of Nigella sativa essential oil. *Phytother Res* 2000; 14: 323-328.

31. Lambert RJ, Skandamis PN, Coote PJ, Nychas GJ. Study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacro. *J Appl Microbiol* 2001; 91: 453-462.
32. Vardar-Unlü G, Candan F, Sökmen A, Daferera D, Polissiou M, Sökmen M, et al. Antimicrobial and antioxidant activity of the essential oil and methanol extracts of *Thymus pectinatus* Fisch. *J Agric Food Chem* 2003; 51: 63-67.
33. Ross C, Morriss A, Khairy M, Khalaf Y, Braude P, Coomarasamy A, et al. A systematic review of the effect of oral antioxidants on male infertility. *Reprod Biomed Online* 2010; 20: 711-723.