

Short Communication

EFFECT OF WATER MELON SEEDS EXTRACTS (*Citrullus vulgaris*) ON SPERMS IN DIABETIC RAT.

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ABSTRACT

Citrullus vulgaris is an antioxidant and has been shown to reduce oxidative stress. Previous studies confirmed that antioxidants have essential effect on infertility through participating in reactive oxygen's species. Chronic hyperglycemia is known to cause infertility in diabetes disease. Wistar male rats (n=40) were allocated into three groups: control group(n=10), *Citrullus vulgaris* seeds extract (CVE) group that received 55mg/kg by gavage method (n=10), and Diabetic group that received 55mg/kg (IP) streptozotocin (STZ) (n=20). The last group was subdivided into two groups of 10. STZ group and treatment group. Treatment group received 55mg/kg (IP) STZ plus 55mg/kg CVE, daily for 4weeks; however, the control group just received an equal volume of (0.9% NaCl) daily (gavage). Diabetes was induced by a single (IP) injection of streptozotocin (55mg/kg). Animals were kept in standard condition. In 28th day, 5cc blood sample was taken from every rat for biochemical analysis. Collecting epididymis tissues, they were prepared for sperm analysis by WHO method. In comparison to other groups, sperm parameters were significantly increased in groups that received 55mg/kg (CVE) (P<0.05). Since in our study, 55mg/kg (CVE) has substantially improving effect on sperm population, so it seems using it by infertile diabetic patients is of beneficial antioxidant effects.

Key words: *Citrullus vulgaris*, Diabetes, Sperm, Rat.

INTRODUCTION

Flavonoids are polyphenolic compounds that are ubiquitous in nature and are categorized, according to chemical structure, into flavonols, flavones, flavanones, isoflavones, catechins, anthocyanidins, and chalcones. Over 4,000 flavonoids have been identified, many of which occur in fruits, vegetables and beverages (tea, coffee, beer, wine and fruit drinks). Because of their potential beneficial effects on human health, flavonoids have aroused considerable interest recently. They have been reported to possess antiviral, anti-allergic, antiplatelet, anti-inflammatory, antitumor and antioxidant activities. Antioxidants are compounds that protect cells against the damaging effects of reactive oxygen species, such as singlet oxygen, superoxide, peroxy radicals, hydroxyl radicals and peroxynitrite (Johnson, 2001). An imbalance between antioxidants and reactive oxygen species results in oxidative stress, leading to cellular damage. Flavonoids may help

in providing protection against diseases by contributing, along with antioxidant vitamins and enzymes, to the total antioxidant defense system of the human body. Epidemiological studies have shown that flavonoid intake is inversely related to mortality from coronary heart disease and to the incidence of heart attacks (khaki *et al.*, 2010). Diabetes is one of the pathological processes known to be related to an unbalanced production of ROS, such as hydroxyl radicals (HO), superoxide anions (O₂) and H₂O₂. Therefore, cells must be protected from this oxidative injury by antioxidant enzymes. Previous study showing impairment by streptozotocin (STZ) of antioxidant enzymes, may contribute to STZ-induced experimental diabetes (Winkler and Moser; 1992). Studies have shown that antioxidants have a widespread effect in andrology, protect spermatozoa from ROS producing abnormal spermatozoa, scavenge ROS produced by leucocytes, prevent from DNA fragmentation, improve semen quality in smokers, reduce

cryodamage to spermatozoa, and finally, block premature sperm maturation. The aim of this study was to see the effect of *Citrullus vulgaris* seeds extract as an antioxidant on sperm parameters and blood antioxidant capacity in diabetic male rats.

Worldwide studies have been done to make use of herbal medicine in different fields of medicine. Base on ancient Persians traditional books, using of herbal medicine has positive effect on treatment of different diseases especially on diabetes mellitus. Several studies have reported that antioxidants and vitamin A, B, C, and E in diet can protect DNA of sperm from free radicals and increase blood-testis barrier stability (Jedlinska-krakowska *et al.*, 2006). Previous study showed that the plant seeds extracts of *Citrullus vulgaris*, *Canna indica*, *Citrus sinensis* and *Capsicum annum* were used for determination of their phenolic and flavonoids contents and for their antioxidant activity by various antioxidant assays (Johnson. 2001).

METHODOLOGY

Animals

Forty adult wistar albino male rats weighing 250 ± 10 g and 8 weeks old were obtained from animal facility of pasture institute of Iran. Male rats were housed in rooms with controlled temperature (25°C), constant humidity (40-70%), and 12h/12h light/ dark cycle prior to use in experimental protocols. All animals were treated in accordance to the Principles of Laboratory Animal Care. The experimental protocol was approved by the Animal Ethical Committee in accordance with the guide for the care and use of laboratory animals prepared by Tabriz medical University. All Rats were fed with a standard diet and water. The daily intake of animal water was monitored at least one week prior to start of treatments in order to determine the amount of water needed per experimental animal. Thereafter, the Wistar male rats ($n=40$) were allocated into three groups: control group ($n=10$), *Citrullus vulgaris* (CVE) group received 55mg/kg by gavage method ($n=10$), and Diabetic group that received 55mg/kg (IP) streptozotocin (STZ) ($n=20$). Diabetic group was subdivided into two groups of 10: STZ group and treatment

group. Treatment group received 55mg/kg (IP) STZ plus 55mg/kg CVE, daily for 4 weeks; however, the control group just received an equal volume of distilled water daily (gavage). Diabetes was induced by a single (IP) injection of streptozotocin (55mg/kg). However, the control group just received an equal volume of (0.9% NaCl) daily. Diabetes was induced by a single intra peritoneal (I.P) injection of streptozotocin (STZ, Sigma- U.S.A.) in 0.1M citrate buffer (pH 4.0) at a dose of 55mg/kg body weight.

Preparation of extract

Citrullus vulgaris fruit were bought in East Azarbyjan province, Tabriz city in Iran. Fifty grams of *Citrullus vulgaris* seeds were removed and dried in room temperature. The powder was obtained through mixing the seeds, and then, extracted once by using 70% methanol. The extracted was left being dried by rotary evaporator.

Induction of experimental type I diabetes

Experimental type I diabetes was induced in rats by intra peritoneal (I.P) injection of 55mg/kg streptozotocin (STZ) in distilled water. Control rats were only received distilled water.

Blood glucose determination

Blood samples were collected from the tail's vein. Basal glucose levels were determined prior to STZ injection, using an automated blood glucose analyzer (Glucometer Elite XL). Sample collections were then made 48h after STZ injection, and blood glucose concentrations were determined and compared between groups. Rats with blood glucose concentrations above 300mg/dL were declared diabetic and used in the experimental group. One week after the induction of experimental diabetes, protocol was started.

Surgical procedure

In the 28th day, the Pentobarbital sodium (40mg/kg) was administered intra peritoneal for anesthesia, and the peritoneal cavity was opened through a lower transverse abdominal incision. There after epididymis, in control and experimental groups, was immediately removed.

Table I. The effect of the 55 mg/kg/rat *Citrullus vulgaris* seeds extracts (CVE) on Blood glucose, Sperm count and epididymis weight of control and experimental groups in the rats.

Samples Groups	Control (n=10)	CVE , (55mg/kg- perday) (n=10)	STZ (55mg/kg(IP) (n=10)	CVE , (55mg/kg- per day + STZ (55mg/kg(IP) (n=10)
Epididymis (gr)	1±0.33	1±0.55	0.99±0.55	1.1±0.55
Blood glucose (mg/dL)	130.3 ±0.33	120.1±0.55*	390.4 ±0.33**	295.5±0.55**
Sperm concentration (total count) (No of sperm/rat '10 ⁶)	38.40±1.29	63.20±2.35*	5**. 0 ±1.20	31.5±0.55

Data are presented as mean ± SE.

*Significant different at P< 0.05 level, (compared with the control group).

**Significant different at P< 0.01 level, (compared with the control group).

The weights of epididymis in each group were registered. The animals were decapitated between 9:00 AM and 11:00 AM.

Epididymis sperm count

Sperms from the cauda epididymis were released by cutting into 2mL of medium (Hams F10) containing 0.5% bovine serum albumin (Feng *et al.*, 2001). After 5min incubation at 37°C (with 5% CO₂), the cauda epididymis sperm reserves were determined using the standard hemocytometric method and analyzed with microscope (Olympus IX70) at 10 field and reported as mean of motile sperm according to WHO method (WHO-1999).

Statistical analysis

Statistical analysis was performed using the ANOVA for comparison of data in the control and experimental groups. The results were expressed as Mean ± S.E.M (standard error of means). P-value less than 0.05 & 0.01 were considered significant and are written in the parentheses. The data were analyzed by SPSS software (release 17).

RESULTS AND DISCUSSION

Weight of Epididymis:

Administration of 55mg/rat *Citrullus vulgaris* seed extract for 28 consecutive days, showed there was no significant difference in epididymis weights between the groups (Table I).

Results of serum blood glucose level

Administration of 55mg/rat *Citrullus vulgaris* seed extract for 28 consecutive days significantly decreased blood glucose in STZ groups as compared to control group (P<0.05).

The blood glucose level was (130.3±0.33)mg/dL in control group, and in CVE, STZ and CVE+STZ groups, it was (120.1±0.55; 390.4±0.33; 295.5±0.55), respectively (Table I).

Results of sperm count

Administration of 55mg/rat *Citrullus vulgaris* seed extract for 28 consecutive days significantly increased Sperm concentrations in herbal groups as compared with the control group. The sperm concentration was (38.40±1.29) in control group, and in CVE, STZ and CVE+STZ groups, it was (63.20±2.35; 20.1±0.5; 31.5±0.55), respectively (Table I).

Data from animal models strongly suggest that diabetes impairs male fertility. Numerous studies have demonstrated a marked reduction in fecundity when male animals are diabetic (Jandric-Balen *et al.*, 2003), as well as an impairment of sperm quality (Mallidis *et al.*, 2009). Animal studies using rodent models of streptozotocin-induced diabetes have demonstrated a reduction in sperm counts and quality (Khaki *et al.*, 2009). In addition, a marked reduction in fecundity has been observed after 15 days, following the injection of

streptozotocin Rehman *et al.*, 2001). Other groups have reported similar findings after longer periods of induced diabetes (Mrowicka, 2001). The associated reduction in fertility is more pronounced when diabetes is induced in pre-pubertal animals (Khaki *et al.*, 2010). Evidence suggests that certain phytochemicals found in citrus sources, such as flavonoids and limonoids, play a major role in treating or retarding chronic diseases, oxidative stress and diabetes (Nasiri *et al.*, 2009). Our experiments demonstrated sperm count was significantly decreased ($P < 0.05$) in diabetic groups; this side effect seems belong to direct toxic effect of STZ or one of its metabolites, having cytotoxic and carcinogenic properties (Kesavulu *et al.*, 2001). Regarding the effect of diabetes on male fertility, in diabetic rats when compared with non-diabetic, results showed promotion in blood glucose levels in STZ-injected rats. Nowadays, the prevention of many diseases has been associated with the ingestion of different plants rich in natural antioxidants (Johnson, 2001; Virgili *et al.*, 2001).

CONCLUSION

The study in *Citrullus vulgaris* seed extract groups indicated serum glucose was significantly decreased and sperm count was increased ($P < 0.05$). It seems all these results are in line with decreasing levels of Reactive oxygen species in serum and cause to balance between ROS and antioxidant defenses and diminish oxidative stress effects (Halliwell, 1993; Khaki *et al.*, 2009). We suggest that flavonoid content of *Citrullus vulgaris* seeds extract administration could be used as antioxidant in food and medicinal sciences. It, also, increases sperm population, reduces blood glucose, shows protective effect in diabetic infertile men, and can give chances in diabetic infertile couple to get new life.

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