Original article

Ameliorative effects of *Spinacia oleracea* on sperm morphology, count, and motility by normalizing the obesity-induced oxidative stress in Sprague Dawley rats

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ABSTRACT

Background: Obesity is a prevailing metabolic disorder that affects the functioning of the male reproductive system. Excessive adipose tissue enhances reactive oxygen species generation and is linked with male infertility. Spinach has demonstrated antioxidant effects. The present study was conducted to determine the antioxidant effects of spinach on sperm parameters in obese Sprague Dawley rats.

Subjects and methods: This randomized control study was conducted at the animal house of the National Institute of Health Islamabad, Islamic International Medical College, Cosmesurge International Hospital, Rawalpindi, and Apollo lab, Islamabad, Pakistan from April 2016 to March 2017. Forty male Sprague Dawley rats having an age of 8 weeks and weight 160-200g were tagged from number 1 to 40. Every third rat was randomly allocated to control Group A (n=13) and remaining into the Experimental group (n=27). Rats of control Group A was given a standard diet while a high-fat diet was given to Experimental group rats to induce obesity for the duration of six weeks. Weight (g) was measured weekly and obesity was confirmed when rats attain more than 20% weight when compared with that of rats of control Group A. Then, after obesity induction, the experimental group was alienated into the obesity control group (Group B) and spinach treated group (Group C). For sample, rats of Group A and Group B were sacrificed, and the cauda epididymis of each rat was placed in a Petri dish containing normal saline and cut into pieces to allow the release of sperm and then sperm parameters (sperms concentration, motility, and morphology) were recorded under the microscope. Then, spinach (5% hot water extract) along with the persistence of fat diet was administered to Group C for 4 weeks and finally, sperm parameters were measured in this group.

Results: Sperm concentration/ml, motility (%), and normal morphology (%) of Group B rats were significantly decreased as compared to Group A rats. However, sperm concentration/ml, motility (%), and normal morphology (%) of Group C (spinach treated group) rats was significantly increased ($p<0.001$) as compared to Group B (obesity control group) rats after administering spinach.

Conclusion: The addition of Spinach in a normal diet regimen restores normal sperm morphology, improves sperm motility and concentration.

Keyword:

Antioxidant; Male infertility; Obesity; Sperm; Spinach

INTRODUCTION

Obesity, which is generally referred to as unnecessary and anomalous accumulation of fat in adipose tissues which has become a global health issue.[1,2] Stated frequency of obesity is thirteen percent globally.[3] Reduced physical activity and use of fat diet are among the two vital contributory factors accountable for weight gain.[4] Obesity, not only the risk issue for different ailments but also accountable for detrimental effects on reproductive functions and fertility by causing hormonal dysregulation and by producing reactive oxygen species.[5,6] In obesity, accretion of adipose tissue causes the production of reactive oxygen species which enhances the generation of cytokines and leads to the cellular grievance.[7] These species reduce the sperm count, motility, and the proportion of sperms with normal morphology by attacking sperms plasma and DNA.[8,9] Antioxidants utilization is a suitable way to diminish the damaging effects of obesity.[10] Currently, there is increasing interest in natural antioxidants-found in herbs and plants as they are cheap, accessible, and have no hazardous side-effects.[11] Spinach is rich in micronutrients-vitamin, folic acid, and oxalic acid and minerals.[12] It also has antioxidants like flavonoids and p-coumaric acid.[13] So spinach can works as armor against the destructive effect of oxidative stress.[11] Large

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number of studies have established the beneficial effects of spinach.[13-16] However, evidence regarding favorable properties of spinach on sperm count, morphology, and motility in obesity is reported less frequently. The present interventional animal study intended to determine the potential ameliorative properties of spinach on sperm count, morphology, and motility.

SUBJECTS AND METHODS

This experimental trial was conducted at animal House of National Institute of Health Islamabad, Islamic International Medical College, Cosmesurge International Hospital, Rawalpindi, and Apollo lab, Islamabad, Pakistan from April 2016 to March 2017. This study was conducted once being approved by the institutional ethical board of Riphah International University, Islamabad. Forty male Sprague Dawley rats, weighing 160-200g were included in the current study. Rats with any other obvious anomaly or with weight above 200g were excluded from the study. Rats were acclimatized for seven days before the commencement of the experimental trial at Animal house. They were provided with a standard diet and water ad libitum.

The standard diet composition of 10 Kg is common salt (0.05 Kg), vitamins/minerals/amino acids (0.10 Kg), soybean oil (0.51 Liter), fish meat (1.50 Kg), dried skimmed milk powder (2 Kg), wheat bran (2.85 Kg) and wheat flour(2.85 Kg). A high-fat diet was prepared by supplementing the standard diet with 20% butter (2 Kg). The supplemented spinach diet was prepared by adding 5% spinach hot water (0.50 Kg) in a high-fat diet. Spinach leaves were bought and got identified by National Herbarium Department, Islamabad. Spinach leaves were washed, macerated, filtered, and then allowed to dry. Spinach powder mixed with distilled water was autoclaved and then the final extract was stockpiled.

After acclimatization, rats were numbered by placing tags on them, from number 1 to 40. Then every third rat was allocated to Group A referred to as the Control group which was provided with a standard diet and the remaining rats in the experimental group were administrated with a high-fat diet to induce obesity in experimental group rats. Weight (g) of the rats was measured weekly by using an electronic weighing machine. After completion of the 6th week, experimental group rats gained 20% more weight as compared to the Group A rats. The experimental group was then subdivided into Group B (obesity control group) and Group C (spinach treated group). The first sampling was done from Group A rats and Group B rats to calculate sperm parameters (sperm concentration, motility, and morphology). For this sampling, rats were sacrificed, and the left epididymis of each rat was dissected out by making a midline incision in the lower abdomen. After dissection, the left cauda epididymis of each rat of Group A and Group B rats was placed in a petri dish containing 2ml of normal saline and cut into pieces to allow the release of sperm. After 15 minutes, 20µl of suspension containing sperms was taken from the petri dish and added into 3980µl of normal saline to dilute it 200 folds. After dilution, sperm concentration/ml, motility (%), and sperms with normal morphology (%) were measured by the Neubauer chamber. Sperm concentration was estimated by placing 10µl of Sperm suspension in the Neubauer chamber under a 40X microscope objective. Neubauer chamber is divided into nine small squares with a large central square. The central large square of the Neubauer chamber has further 25 squares but sperms were counted in 5 of these 25 small squares. These 25 small squares have a volume of the 0.1µl. As sperms were counted in 5 square, it means only those sperms were counted that were settled in the 0.02µl (0.1/5=0.02). Thus, sperm count in 5 squares was multiplied by 50,000 to determine the number of sperms in 1ml. The final count was then multiplied by the dilution factor and the results were expressed as sperm/ml. For determining the sperm motility, 10µL of sperm suspension was placed on a slide with a cover glass. A total of 100 sperms were observed for motility under the microscopic field at the magnification of 40x and the results were expressed as a percentage of motile sperms of the total sperms. Sperm suspension was prepared by adding an eosin stain drop. A drop of sperm suspension was spread on a clean slide, which was observed for sperm morphology after getting air-dried. One hundred sperms were randomly assessed at the magnification of 40x and the results were expressed in terms of the percentage of sperms with normal morphology. After the first sampling, Group C rats were provided with 5% spinach hot water extract in addition to a high-fat diet for four weeks. The second sampling from spinach treated Group C rats was done to calculate sperm parameters (sperm concentration, motility, and morphology) at the end of the 4th week.

Statistical analysis was done by using the Statistical Package of social sciences version 21. An independent sample t-test was applied to compare groups while considering a p-value of <0.05 as significant. All results were expressed as mean $\pm$ SEM.

RESULTS

Comparison of Mean ± SEM of sperm concentration, sperm motility, and sperm with normal morphology of all three groups is shown in Table 1. Sperm concentration of obese Group B rats (19.80 ± 0.55 ×106/ml) was significantly decreased as compared to control Group A rats (28.50 ± 0.56 ×106/ml). While sperm concentration of spinach treated obese Group C rats (35.30 ± 0.83 ×106/ml) was significantly increased (p<0.001) as compared to obese Group B rats (19.80 ± 0.55 ×106/ml) after giving spinach extract. Motility of obese Group B rats (32.30 ± 0.65 %) was significantly decreased (p<0.001) than normal Group A rats (59.10± 0.93 %). Sperm motility was significantly increased (p<0.001) in spinach treated obese Group C rats (42.50 ± 2.14 %) after administration of spinach hot water extract when compared with obese Group B rats (32.30 ± 0.65 %). The percentage of sperms with normal morphology was significantly reduced (p<0.001) in obese Group B rats (34.50 ± 1.38 %) as compared to control Group A rats (95.90 ± 0.58 %). In comparison, the normal morphology percentage of sperms in spinach treated obese Group C rats (79.50 ± 1.38 %) showed significant improvement (p<0.001) when compared to obese Group B rats (34.50 ± 1.38 %) after giving spinach extract.

Table 1. Comparison of Mean ± SEM of sperm parameters (sperm concentration, motility, and morphology) levels of Sprague Dawley rats in all three groups

Sperm Parameters	Group A Control *n*=13	Group B Obesity control group *n*=13	Group C Spinach treated group *n*=14
Sperm concentration (10^6/ml)	28.5 ± 0.56	19.8 ± 0.55***a	35.3 ± 0.83***b
Sperm motility (%)	59.1 ±0.93	32.3± 0.65***a	42.5± 2.14***b
Normal sperm morphology (%)	95.5 ± 0.58	34.5 ± 1.38***a	79.5 ± 1.38***b

**a: Group A *vs.* B; **b: Group B *vs.* C

DISCUSSION

Obesity is an extreme ubiquitous metabolic ailment that has turned out to be a prodigious challenge throughout the world.[17,18] This metabolic condition is closely linked with numerous ailments such as endocrinal abnormalities, insulin resistance, hypertension, and other cardiovascular diseases.[19] Besides, mounting evidence advocates that along with other illnesses, obesity also distresses the functioning of the male reproductive system and is linked with male infertility.[19,20]

The current study revealed the deleterious effects of obesity on sperm parameters and the use of *Spinacia oleracea* in reinstating fertility. A comprehensive analysis of sperm parameters was conducted in the present study to identify the abnormalities that linked obesity with male infertility. Remarkably, obese rats, when compared with control rats, demonstrated abnormal sperm morphology in addition to decreased sperm motility and concentration. One of the explanations of decreased sperm concentration can be the overproduction of free radicals which attacks the vital component of sperm. Furthermore, the cell membrane of sperm is composed of polyunsaturated fatty acids and is very vulnerable to lipid peroxidation caused by free radicals. This process of lipid peroxidation causes an interruption in ATP production and it enhances membrane permeability, one of the reasons for the decline in sperm motility. Similarly, change in sperm morphology can occur due to alteration in the axoneme structure of sperms by free radicals. Similar findings have been observed by previous researchers.[21,23] However, this study shows that spinach (*Spinacia oleracea*) can restore the ameliorative effects of obesity on sperm parameters including sperm motility and sperm concentration. In addition to that, spinach (*Spinacia oleracea*) also elevated the number of sperms with normal morphology. These results are in agreement with the findings of Mortazavi and coauthors who revealed that usage of vitamin C and E has positive beneficial effects on sperm concentration, motility, and the number of sperms with normal morphology in obese rats.[24]

Results of the present study establish that oxidative stress is provoked by obesity and current findings are in line with former studies.[23,25] However, the salutary use of spinach, a natural antioxidant can repair oxidative damage and improves the functions of the male reproductive system.

CONCLUSION

Spinacia oleracea improves normal sperm morphology, motility, and concentration. It has restorative and antioxidant effects on the male reproductive system. It encounters obesity-induced oxidative stress changes in the male reproductive system.

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