ORIGINAL ARTICLE

Antioxidant Effects of Spinach (Spinacia Oleracea) on Testicular and Epididymal Weight of Obese Sprague Dawley Rats

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ABSTRACT

Objective: To determine the antioxidant effects of spinach on the weight of testes and epididymis in obese Sprague Dawley rats.

Study Design: Experimental, randomized control study.

Place and Duration of Study: It was carried out from April 2016 to March 2017 in the department of physiology of Islamic International Medical College, Rawalpindi, Pakistan.

Materials and Methods: Total 40 male Sprague Dawley rats of about 8 weeks were included. They were randomly divided into Group A- Fed on standard diet and Experimental group- Fed on high fat diet to induce obesity. At the end of 6th week, after inducing obesity, experimental group was subdivided into Group B and Group C. Weight of the testes and epididymis of Group A and Group B rats was recorded. Then 5% spinach was given to Group C along with high fat diet for 4 weeks and finally weight of the testes and epididymis was measured.

Results: Weight of the testes and epididymis (g) of Group B (1.32 ± 0.53g) rats was significantly decreased (P<0.001) as compared to Group A (1.88 ± 0.92g) rats. However, weight of testes and epididymis (g) of Group C (1.92 ± 0.49g) rats was significantly increased (P<0.001) as compared to Group B (1.32 ± 0.53g) rats after spinach intake.

Conclusion: Intake of spinach supplemented diet has ameliorative effects on weight of testes and epididymis in response to deleterious effects caused by obesity-induced oxidative stress.

Key Words: *Epididymis, Obesity, Spinach, Testes.*

Introduction

Obesity, which is generally referred as anomalous and unnecessary accretion of fats in adipose tissues has become an intricate health problem.[1,2] Reported prevalence of obesity is 13% worldwide.[3] The reported prevalence of obesity in Pakistan is 10.3%.[4]

Different contributory factors are responsible for increase in weight but high fat diet and decrease physical activity are the two fundamental causes.[5] Obesity is not only the risk factor of different diseases but also accountable for harmful effects on reproductive system and increase in the incidence of infertility.[6,7]

Infertility is a global health problem, as 15% to 20% couples struggles when trying to conceive and hence look for medical guidance to increase the possibility of successful pregnancy.[8] For proper functioning of male reproductive system, testes and epididymis serves as an imperative organ as they are main site of sperm production and maturation respectively.[9]

One of the links connecting obesity with infertility is generation of reactive oxygen species.[10] In obesity, increase release of cytokines and cellular injury due to the pressure effect caused by excessive adipose tissues resulting in increased production of reactive oxygen species.[11] These reactive oxygen species are extremely unstable that gain stability by attacking the adjacent stable species, resulting in damage to surrounding cells and tissues.[12]

Thus, antioxidants utilization may be appropriate way to lessen the harmful effects of obesity.[13] These antioxidants act as scavenger against reactive oxygen species and help to overcome the unwanted consequences of obesity on male reproductive system. Currently, there is extremely curiosity

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regarding natural antioxidants found in plants as they are affordable and easily accessible and have no perilous effects.[14]

Spinach is green flourishing vegetable. It is rich in micronutrients (vitamin A, B, E, C and folic acid and oxalic acid) and minerals (calcium, phosphorus, iron, zinc, potassium and sodium).[15] It is also an established source of flavonoid and p-coumaric acid all of which have antioxidant properties.[16] So spinach can works as a shield against the damaging effect of reactive oxygen species.[14]

A number of studies on different animal models have demonstrated the antioxidant effects of spinach such as its effects on hyperlipidemia, radiation-induced biochemical changes in testes, lipopolysaccharides induced endotoxemia and slowing tumorigenic progression in prostatic carcinoma.[16,19] However, its therapeutic effects on male infertity induced by obesity are still needed to be explored and information regarding curative effect of spinach on obesity-induced changes on weight of testes and epididymis (sex organs) is still required. Thus, this will enable us to recommend this nutritional plant in the diet course of obese and overweight population to avert and alleviate these changes. As a result, the present study was intended to determine the potential effects of spinach on weight of sex organs.

Materials and Methods

The experimental randomized control study was performed in the department of Physiology and multidisciplinary research laboratory of Islamic International Medical College, Rawalpindi in association with the Animal House at National Institute of Health (NIH), Islamabad, Pakistan from April 2016 to March 2017. The study was approved by the ethical review committee and was accomplished under the guidelines, stated by the National Institute for animal experimentations.

A total of 40 male Sprague Dawley rats of about 8 weeks weighing from 160-200g were included in the study. For seven days, rats were allowed to acclimatize to NIH Animal house environment at humidity of 50-70% and at a room temperature of 24 + 2 °C, maintained at a 12 hour light and dark cycle. A standard diet in pellet form was prepared at the Animal house of NIH, Islamabad. The food and water was provided ad libitum.

Diet protocol

Spinach (Spinacia oleracea) leaves were bought from local market, and identified by taxonomist of National Herbarium department in Plant Genetic Resources Institute and National Agriculture Research Centre (NARC) Islamabad, allotting voucher number # IIMC 05.

Fresh spinach leaves were washed thoroughly with distilled water and then crushed in electric blender. The macerate was then filtered and air dried. Spinach powder was mixed with distil water and autoclaved at 121°C temperature and 15 Ibs pressure and then extract was stored.[17] Then 500 gram of this extract was added into 10kg of standard diet. Therefore, supplemented spinach diet contained standard diet along with 5% spinach hot water extract. Composition of standard and spinach supplemented diet is shown in Table I.

The composition of high fat diet constituted standard diet supplemented with 20% butter as shown in Table I.

Table I: Composition of standard diet, high fat diet and spinach supplemented diet.

Ingredients	Standard diet	High fat diet	Spinach supplemented diet
Wheat bran	2.85(kg)	2.85(kg)	2.85(kg)
Wheat flour	2.85(kg)	2.85(kg)	2.85(kg)
Dried skimmed milk powder	2.00(kg)	2.00(kg)	2.00(kg)
Fish meat	1.50(kg)	1.50(kg)	1.50(kg)
Common salt	0.05(kg)	0.05(kg)	0.05(kg)
Vitamins/minerals/amino acids	0.10(kg)	0.10(kg)	0.10(kg)
Butter		2(kg)	2(kg)
Spinach			500(gram)
Soybean oil	0.51 (Liter)	0.51(Liter)	
Total weight	10 kg	12(kg)	12.5(kg)

After acclimatization, rats were randomly divided into Group A (Control group n=13) and Experimental group (n =27). Group A rats were fed on standard diet and rats of Experimental group were fed on high fat diet to induce obesity in six weeks. Weight (g) of the rats was measured weekly by placing them on the weighing machine (TS200 electronic compact scale, Jiangyin Ditai electronic technology Co. Ltd., China).

At the end of 6th weeks, when rats of Experimental group had gained 20% weight above that of rats of

Group A (Control group), they were further subdivided into Group B (Obesity control group n =13) and Group C (Spinach treated group n=14).Then first sampling for measuring weight of testes and epididymis from Group A (Control group) and Group B (Obesity control group) Sprague Dawley rats was done by placing them in a glass jar containing cotton soaked in chloroform. The rats were kept there, until their breathing movements ceased and they were sacrificed. Then an incision was given in the abdominal wall along the midline and the right testes and epididymis were dissected out, freed from adherent tissues and weighed (g) on weighing machine (TS200 electronic compact scale, Jiangy in Ditai electronic technology Co. Ltd., China).

Then 5% spinach hot water extract was given to Group C (Spinach treated group) along with continuation of high fat diet for four weeks. At the end of 4th week, second sampling to calculate weight of testes and epididymis was done from Group C (Spinach treated group) Sprague Dawley rats similar to the method applied for the first sample collection.

Statistical analysis was performed by using Statistical package of social sciences (SPSS 21) version. All Results were expressed as Mean ± SEM. Comparisons among the groups was evaluated by using the independent sample t-test. P value of <0.05 was regarded as significant.

Results

Weight of testes and epididymis (g) of Group B rats (1.32 ± 0.53g) was significantly decreased (P<0.001) as compared to Group A rats (1.88 ± 0.92g). However, weight of testes and epididymis of Group C rats (1.92 ± 0.49g) was significantly increased (P<0.001) as compared to Group B rats (1.32 ± 0.53g) after administration of spinach hot water extract. Comparison of Mean ± SEM of weight of testes and epididymis (g) of all three Groups (A, B, C) is shown in Figure 1.

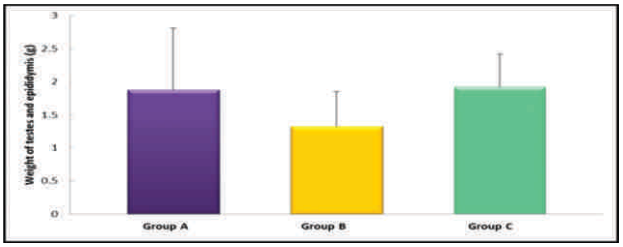

Fig 1: Comparison Of Mean ± SEM Of Weight Of Testes And Epididymis (G) Of Sprague Dawley Rats In The Following Three Groups:

Group A: Control group
Group B: Obesity control group
Group C: Spinach treated group
***=P < 0.001 is considered statistically highly significant.
***a= Group A vs B
***b= Group B vs C

Discussion

Obesity has become a great challenge for both developed and developing countries of the world.[20] Growing evidence suggests that along with other diseases, increased body fat also affect the functions of male reproductive system and cause male infertility.[21,22] Thus, our study explored the harmful effects of obesity on weight of testes and epididymis as well as the beneficent role of spinach (*Spinacia oleracea*) in restoring these changes.

In obesity, there is not only increased fat accumulation in different parts of the body but also around testes and epididymis as described by Alhashem et al. (2014).[23] These excessive adipose tissues cause cellular damage resulting in increased production of reactive oxygen species and expose the testicular microenvironment to oxidative stress. Our study also shows that obesity causes considerable decrease in the weight of testis and epididymis. Similar results have been reported by Alhashem et al., (2014) and Yan et al., (2015) that after inducing obesity, there was significant decrease in testes and epididymis weight when compared with control.[23,24] However, Ghanayem, Bai, Kissling, Travlos & Hoffler, 2010 had different observations.[25] Reason of those variations can be due to difference in genetics of animal model as well as procedure differences.

However, use of antioxidant such as spinach (*Spinacia oleracea*) could repair the oxidative damage and cause significant increase in weight of testes and epididymis in obese rats. These results are in accordance with the study carried out by Tawfeek, Ahmed & Kakel, (2006) who observed the antioxidant effect of Nigella sativa oil treatment on the sex organs in rats exposed to oxidative stress and concluded that use of antioxidant could repair the damage caused by oxidative stress.[26] Similarly, Elgazar, (2016) also showed that use of antioxidants have beneficial effect on the testicular tissue and cause significant increase in weight of sex organs

while describing the protective role of walnut seeds extract and vitamin E as an antioxidant against the oxidative stress.[27] Although we demonstrated that obesity induce injurious effects on weight of testes and epididymis, further biochemical and molecular studies are needed to clarify the effects of obesity and spinach (*Spinacia oleracea*) on the male reproductive system. Furthermore, these therapeutic effects of spinach (*Spinacia oleracea*) need to be clarify via future studies on human.

Conclusion

Thus our study shows that spinach has strong potential as an antioxidant to restore the deleterious effects on weight of testes and epididymis in response to obesity-induced oxidative stress.

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Finding source

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