Effect of *Hibiscus sabdariffa* on sperm quality and testicular oxidative stress in rats fed with high fat diet

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Abstract

Obesity, a condition of abnormal fat accumulation in adipose tissue, is a major risk factor for deleterious associated pathologies such as insulin resistance, type 2 diabetes and male infertility. Several studies have shown the positive correlation between obesity and oxidative stress and its effect on the male reproductive system. Roselle (*Hibiscus sabdariffa*) contains phenolic compounds, especially anthocyanins which act as antioxidants. The objective of this study was to evaluate the effect of roselle on sperm quality and oxidative stress in the testes of rats fed a high fat diet. The rats were divided into 4 groups (n = 6): control group fed with a normal diet, rats fed with a high fat diet (HFD), rats fed with a high fat diet treated with 250 mg/kg body weight roselle (HFD-L), and rats fed with a high fat diet treated with 500 mg/kg roselle (HFD-H). Eight weeks after administration, sperm were collected from rat epididymis and the sperm quality and morphology were assessed. The rats' testes were assessed for malondialdehyde (MDA) levels. Sperm concentration, motility and normal morphology showed a significant increase in HFD-L and HFD-H groups when compared with HFD group ($P < 0.05$). Sperm viability was not significantly different among experimental groups ($P > 0.05$). MDA levels in the HFD group showed a significant increase when compared with the control ($P < 0.05$). MDA levels in the HFD-L and HFD-H groups were significantly decreased when compared with the HFD group ($P < 0.05$). These results show that roselle reduced oxidative stress and increased sperm quality, which clearly demonstrate that roselle has the beneficial effects on sperm quality in rats fed with high fat diet.

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Introduction

Obesity occurs as an accumulated excess amount of body fat. It is a risk factor for deleterious associated pathologies, such as hypertension, cardiovascular disease,[1] type 2 diabetes associated with insulin resistance, and male infertility.[2,3] Numerous studies confirmed that obesity is the cause of various changes including oxidative stress,[4] inflammation,[4] mitochondrial dysfunction,[5] and apoptosis.[6] These changes encourage many pathophysiological conditions in the body.

The increase in fat accumulation, especially white adipose tissue, leads to the production of adipokines[7] involving the secretion and generation of free radicals (reactive oxygen species, ROS). Adipokines and ROS are important mediators for inducing oxidative stress.[4] Lipid peroxidation is a process whereby oxygen reacts with unsaturated lipids, especially polyunsaturated fatty acid (PUFA), producing mixtures of lipid hydroperoxide and aldehydic end-products, such as malondialdehyde (MDA). This reaction occurs in several cell types, including sperm cells, particularly in the plasma membrane which contains a high lipid content. Sperm cells are sensitive to oxidative stress. Lipid peroxidation can affect sperm cells by decreasing sperm concentration, motility, viability, and normal morphology.[3,8,9] In a normal physiological state, antioxidant enzyme mechanisms can alleviate these ROS and protect the spermatozoa against any likely damage. However, high ROS levels in the obese may diminish antioxidant mechanisms resulting in oxidative stress. Emerging research evidence has suggested that antioxidant can attenuate the effects of oxidative stress from obesity.[10] In addition, treatment with antioxidants or ROS inhibitors could restore adipokine regulation.[11] Antioxidants act as free radical scavengers to protect spermatozoa against ROS[12] and compensate for the loss of cytoplasmic antioxidant molecules in sperm during the cytoplasm removal stage in spermiogenesis.[13] Therefore, supplementation with antioxidants could reduce the risk of complications related with obesity and oxidative stress.[14]

Roselle (*Hibiscus sabdariffa*) has been used traditionally in herbal medicines for antibacterial,[15] antihypertensive,[16] antidiabetic,[17,18] antiinflammatory,[19] or anticancer effects[20] and Alzheimer's disease prevention.[21] Previous studies have reported that

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roselle could decrease the levels of total lipids, cholesterols and triglycerides, suggesting an antiobesity effect.[22] Anthocyanin pigments, an antioxidant agent, in roselle have been confirmed as beneficial in various cells by attenuating the negative effects of oxidative stress.[23,24] Previous studies have shown that roselle aqueous extract ameliorates sperm defects in streptozotocin-induced diabetic rats.[25,26] In addition, roselle could protect cisplatin-induced testicular damage and oxidative stress, and improve sperm quality, by decreasing the MDA level.[27]

The purpose of this study was to investigate the effects of *H. sabdariffa* on the sperm functions as well as oxidative stress in the sperm and testes in high fat diet-induced obese rats.

Materials and Methods

Chemicals

Phosphate buffered saline (PBS), sodium dodecyl sulphate (SDS), acetic acid solution, thiobarbituric acid (TBA), 1,1,3,3- tetramethoxypropane (TMP) and nigrosin were purchased from Sigma–Aldrich (St Louis, MO, USA). Pierce™ BCA protein assay kit was purchased from Thermo Fisher Scientific (Waltham, MA, USA) and Diff-Quik staining kit was purchased from RVL supply (Thailand).

Plant extract

One hundred milligrams of roselle powder mixed with 1 ml of distilled water was freshly prepared before use.

Animals and experimental design

All animal protocols were approved from the Research Ethic Committee on Animal Study, Naresuan University, Thailand (NU-AE 580713). Four-week-old male Sprague Dawley rats were purchased from Nomura Siam International, Bangkok, Thailand. Rats were housed at the Center for Animal Research, Naresuan University, under a standard light-dark cycle, constant humidity, and controlled temperature (22 ± 1°C). Twenty-four rats were used in this study. They were divided into four groups of six animals each (n = 6/group). The first group was used as the control and fed a normal diet. The second group was fed a high fat diet (HFD) by gavage, while the third was fed a high fat diet supplemented with roselle 250 mg/kg body weight (BW)/day (HFD-L), and the fourth was fed a high fat diet supplemented with roselle 500 mg/kg BW/day (HFD-H). This process took place over an eight-week period. At the end of the experimental period, rats were anesthetized using thiopental 50 mg/ kg BW before their testes, epididymis, and vas deferens were removed and weighed. The left testis was dissected and kept in 4% formaldehyde and the right testis was dissected and stored at -80°C. Sperm were obtained for sperm quality analysis. Relative testicular weight was calculated by dividing with body weight.

Sample collection and preparation

The epididymis and vas deferens were squeezed using forceps in a 4-well plate containing 1 ml PBS at 37°C. The testicular tissues were homogenized in 1 ml PBS at 37°C and collected in microcentrifuge tubes. The samples were centrifuged at 3500g at 4°C for 20 minutes and the supernatants were collected and stored at -80°C until analysis.

Sperm concentration and motility

Sperm concentration and motility were examined by loading 10 µl of the sperm suspension on a Makler counting chamber and observed under light microscope. A total of 200 sperm were counted for motility and categorized as progressive, non-progressive motility, or non-motile, and reported as percentage of sperm motility. Sperm concentration and motility were evaluated according to methods described by the World Health Organization (WHO).[28]

Sperm viability

Sperm viability was evaluated using eosin-nigrosin staining technique and was carried out according to WHO standards.[28] Eosin (1%) and 10% nigrosin was prepared in distilled water. First, 10 µl sperm suspension were mixed with 1% eosin. After 30 seconds, an equal volume of nigrosin was added to this mixture and then smears were prepared and observed under light microscopy at 400x magnification. Two hundred sperm were counted and the percentage of viable sperm was calculated; viable sperm remained colorless (unstained heads) while nonviable sperm stained pink or red.

Sperm morphology

To study sperm morphology, 10 µl semen was spread onto a glass slide and allowed to air-dry at room temperature. The smears were then stained with Diff-Quik staining kit and sperm morphology was assessed according to WHO criteria.[28] Two hundred sperm were counted and the percentage of normal and abnormal sperm morphology were evaluated at 400x magnification. An abnormal sperm morphology included defects in the neck, midpiece, and tail.

Thiobarbituric acid reactive substance (TBARS) assay

TBARS was expressed in terms of MDA equivalents. Testes were placed into an ice-cold PBS. A mixture of standard or sample, 8.1% SDS, 20% acetic acid solution, and 0.8% TBA were placed in a centrifuge tube and vortexed. The mixtures were incubated at 95°C for one hour and were then added into each well of a 96-well plate. All reactions were done in duplicate. Absorbance was read at 532 nm on a microplate reader. TBARS results were expressed in nmol/mg protein.

Statistical analysis

Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) software.

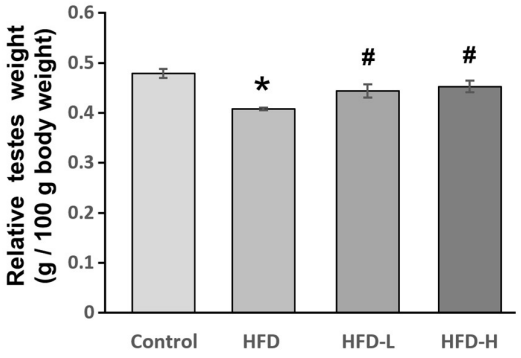

Figure 1 Effects of *H. sabdariffa* extracts on testicular relative weights in high fat diet-induced obesity in rats. Data were expressed as mean ± SEM; n = 6/group. *P < 0.05 compared to control group; #P < 0.05 compared to HFD group.

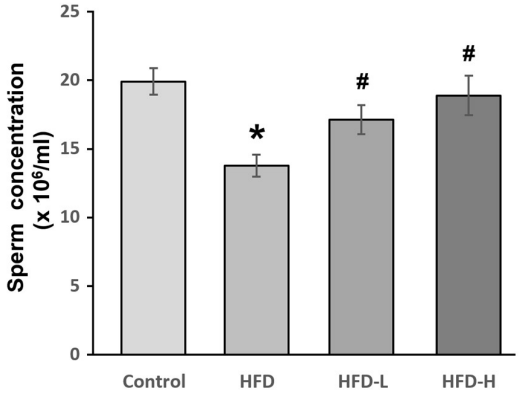

Figure 2 Effects of *H. sabdariffa* extracts on sperm concentration in high fat diet-induced obesity in rats. Data were expressed as mean ± SEM, n = 6/group. *P < 0.05 compared to control group; #P < 0.05 compared to HFD group.

Differences among experimental groups were assessed using one-way ANOVA and were analyzed with Least Significant Difference (LSD), at a significance level (P value) of < 0.05.

Results

Effect of roselle on relative testes weight

The body weight in the HFD group was significantly increased when compared among the control, HFD-L and HFD-H groups. The actual testicular weight was not significantly different among the experimental groups. However, the relative testes weight in the HFD group (0.41 ± 0.003 g/100 g BW) was significantly decreased compared with the control (0. 47 ± 0. 009 g/100 g BW) (P < 0.05). In comparison with HFD group, administration of 250 and 500 mg/ kg body weight roselle resulted in significantly higher relative testes weights (0.44 ± 0.013 and 0.45 ± 0.012 g/100 g BW, respectively; P < 0.05). (Figure 1)

Effect of roselle on sperm concentration

The sperm concentration in the HFD group (13.77 ± 0.79 × 10^6 /ml) was significantly decreased when compared with the control group (19.92 ± 0.98 × 10^6 /ml; P < 0.05). The sperm concentration in HFD group was significantly higher than that of HFD-L (17.13 ± 1.05 × 10^6 /ml) and HFD-H (18.9 ± 1.41 × 10^6 /ml) groups (P < 0.05). (Figure 2)

Effect of roselle on sperm motility

The percentage of sperm motility in the HFD group (37.40 ± 0.79%) was significantly decreased compared with the control group (51. 77 ± 0.98%) (P < 0.05). The percentages of sperm motility were significantly increased in HFD-L and HFD-H groups (47.87 ± 1.05% and 46.36 ± 1.41%, respectively) when compared with the HFD group (P < 0. 05). (Figure 3)

Effect of roselle on sperm viability

There were no statistically significant differences in the percentage of sperm viability among the experimental groups (control, 79.67 ± 0.03%; HFD, 59.29 ± 0.02%; HFD-L, 66.48 ± 0.02%; and HFD-H, 71.73 ± 0.04%, respectively). (Figure 4)

Effect of roselle on sperm morphology

The percentage of normal sperm morphology in the

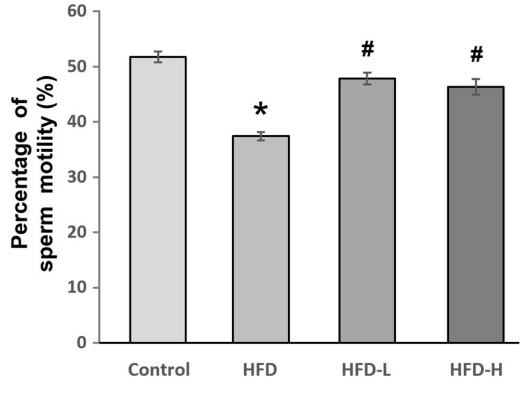

Figure 3 Effects of *H. sabdariffa* extracts on the percentage of sperm motility in high fat diet-induced obesity in rats. Data were expressed as mean ± SEM, n = 6/group. *P < 0.05 compared to control group; #P < 0.05 compared to HFD group.

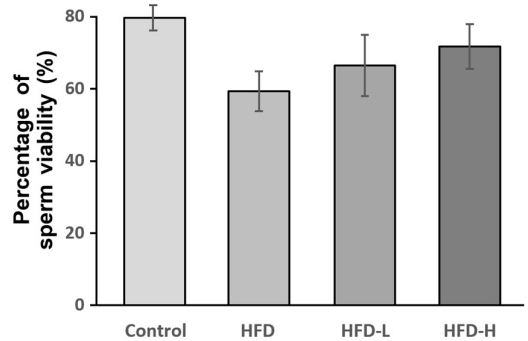

Figure 4 Effects of *H. sabdariffa* extracts on the percentage of sperm viability in high fat diet-induced obesity in rats. Data were expressed as mean ± SEM, n = 6/group.

Table 1 Sperm morphology

	Control	HFD	HFD-L	HFD-H
Normal, %	89.4 (0.5)	71.7 (4.7)*	81.6 (0.5)#	80.9 (0.3)#
Abnormal, %				
Head defect	2.8 (0.03)	6.9 (0.02)	3.1 (0.02)	4.0 (0.04)
Middle piece defect	0.9 (0.2)	1.0 (0.1)	0.3 (0.06)#	0.5 (0.1)#
Tail defect	7.3 (1.0)	16.2 (2.0)*	8.0 (1.0)#	8.0 (0.9)#

Data were expressed as mean (SEM), n = 6/group. *P < 0.05 compared to control group; #P < 0.05 compared to HFD group

HFD group (71.7 ± 4.7%) was significantly decreased when compared with the control group (89.3 ± 0.5%) (P < 0.05). In comparison with HFD group, the HFD-L and HFD-H groups (81.6 ± 0.5% and 80.9 ± 0.3%, respectively) had significantly increased percentage of normal sperm morphology (P < 0.05).

The percentage of head defects were not significantly different among all experimental groups (P > 0.05). The percentage of middle piece defects in the HFD-L (0.3 ± 0.06%) and HFD-H (0.5 ± 0.1%) were significantly decreased compared with the HFD group (1.0 ± 0.1%; P < 0.05). In addition, the percentage of tail defects in the HFD group (16.2 ± 2.0%) was significantly increased compared with the control group (7.3 ± 1.0 %; P < 0.05), whereas the HFD-L and HFD-H (8.0 ± 1.0% and 8.0 ± 0.9%, respectively) groups had significantly decreased percentage of tail defects (P < 0.05). (Table 1)

Effect of roselle on testicular MDA levels

The MDA levels in the HFD group (3.18 ± 0.54 nmol/mg protein) was significantly increased compared with the control group (0.71 ± 0.16 nmol/mg protein; P < 0.05). In comparison with HFD group, administration of 250 and 500 mg/kg body weight roselle significantly decreased the MDA levels (1.19 ± 0.07 and 0.97 ± 0.09 nmol/mg protein; P < 0.05). These results demonstrated the antioxidative activity of roselle. (Figure 5)

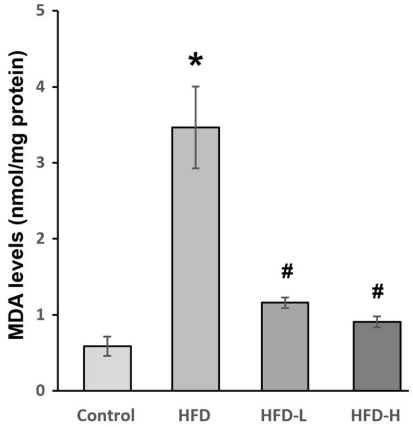

Figure 5 Effects of *H. sabdariffa* extracts on MDA levels in high fat diet-induced obesity in rats. Data were expressed as mean ± SEM, n = 6/group. *P < 0.05 compared to control group; #P < 0.05 compared to HFD group.

Discussion

The obesity prevalence has grown to concern in developing countries in the recent years. It can affect physiological functions and lead to many disorders and diseases, including male infertility. It has been reported that BMI is correlated with semen quality and sperm concentration.[29] Altered semen parameters ascribed to obesity include decreased sperm concentration, abnormal morphology, compromised chromatin integrity, and abnormal motility.[30] Obese men exhibit elevated aromatase activity and secrete adipose-derived hormones including adipokines then tending to present with elevated estrogen and low testosterone and FSH levels.[31] Hypogonadism found in males who are obese can account for problematic spermatogenesis and sperm quality and may contribute to infertility.[32] Diet-induced obesity in mice caused a significant reduction in male fertility and resulted in a fivefold increase in leptin levels compared with control mice.[33] Sperm from these obese males exhibited decreased motility and reduced hyperactivated progression compared with lean mice.[33] Adipocytes secrete various adipokines, for example, tumor necrosis factor α (TNFα), interleukin 6 (IL-6), and plasminogen activator inhibitor-1).[34] They increased the release of adipokines from excess white adipose tissue, resulting in inflammation and possible toxic effects on spermatozoa through the release of excess ROS and RNS.[35]

Roselle is traditionally used as folk and herbal medicines. It was found that roselle had flavonoid compounds containing anthocyanins, which are red substances.[36] However, not many studies determine its benefits on male fertility. Therefore, we considered it interesting to evaluate the effect of roselle extract on the animal model of obese rats. In the present study an increased lipid peroxidation was observed in testes of HFD group but the administration of roselle prevented the increased tissue lipid peroxidation in HFD-L and HFD-H groups. The testicular tissues and spermatozoa are very sensitive to ROS attack that leads to oxidative stress and lipid peroxidation. Oxidative stress impaired sperm membrane and resulted in less sperm motility.[29] The decrease in the relative testicular weight in HFD rats suggests that spermatogenesis in seminiferous tubule and sperm morphology could also have been impaired. It has been reported that high fat diet consumption resulted in the spermatogenesis impairment and affected sperm quality.[29] These changes may alter testicular functions and it was also demonstrated in our present study that high fat diet induced obesity can be an important causative factor in the etiology of male infertility. The data presented here showed that administration of roselle, had multiple beneficial effects including protection against loss of relative testicular weight associated with high fat diet-induced obesity. Our results are similar to those reported by another group of researchers.[37] Excessive fat accumulation in obesity

induces oxidative stress in tissues, resulting in malfunction of reproductive systems. Decreased testosterone levels and altered testicular functions caused by oxidative stress consequently lead to male infertility.[38] The MDA lowering effect in testes was seen in HFD-L and HFD-H groups in the present study. It indicates that roselle extract exhibits an antioxidant capacity against HFD-induced obesity.[38] The relation between oxidative stress and sperm damage was reported in an experimental study. The mechanisms underlying the protective effect of roselle on male reproductive function of rat might be due to its antioxidant effect.[27] Many studies have reported that antioxidants can improve fertility. Roselle rich in antioxidants increased sperm counts, motility, and viability, and enhanced sperm morphology. Treatment of antioxidants like anthocyanins restored sperm motility of cisplatin-treated rats.[27] Moreover, roselle ameliorated carbamazepine-induced oxidative stress responses in the testes of Wister rats. Therefore, roselle has an antioxidant protective effect on fertility. Roselle has a potential protective role against diabetes-induced sperm damage.[37] High dietary fat intake leads to the development of oxidative stress. Roselle showed efficient antioxidant activity by scavenging lipid peroxidation, resulting in decreased oxidative damage to the tissues.

Conclusion

Roselle administration reduce lipid peroxidation as well as improve sperm quality such as sperm concentration, motility, viability and morphology. Thus, the protective role of roselle against high fat diet-induced obesity may be used as an alternative way in the treatment of obesity caused by oxidative stress.

Acknowledgments

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Conflict of Interest

The authors have no conflict of interest.

References

1. Lerman JB, Parness IA, Shenoy RU. Body weights in adults with congenital heart disease and the obesity frequency. Am J Cardiol. 2017; 119: 638-42.
2. Hammoud AO, Gibson M, Peterson CM, Meikle AW, Carrell DT. Impact of male obesity on infertility: A critical review of the current literature. Fertil Steril. 2008; 90: 897-904.
3. Lavranos G, Balla M, Tzortzopoulou A, Syriou V, Angelopoulou R. Investigating ROS sources in male infertility: A common end for numerous pathways. Reprod Toxicol. 2012; 34: 298-307.
4. Fernandez CDB, Bellentani FF, Fernandes GSA, Perobelli JE, Favareto APA, Nascimento AF, et al. Diet-induced obesity in rats leads to a decrease in sperm motility. Reprod Biol Endocrinol. 2011; 9: 32. doi: 10.1186/1477-7827-9-32.
5. Bournat JC, Brown CW. Mitochondrial dysfunction in obesity. Curr Opin Endocrinol Diabetes Obes. 2010; 17: 446-52.
6. Kluth O, Mirhashemi F, Scherneck S, Kaiser D, Kluge R, Neschen S, et al. Dissociation of lipotoxicity and glucotoxicity in a mouse model of obesity associated diabetes: Role of forkhead box O1 (FOXO1) in glucose-induced beta cell failure. Diabetologia. 2011; 54: 605-16.
7. Fantuzzi G. Adipose tissue, adipokines, and inflammation. J Allergy Clin Immunol. 2005; 115: 911-9.
8. Sies H. Oxidative stress: Oxidants and antioxidants. Exp Physiol. 1997; 82: 291-5.
9. Du Plessis SS, Cabler S, McAlister DA, Sabanegh E, Agarwal A. The effect of obesity on sperm disorders and male infertility. Nat Rev Urol. 2010; 7: 153-61.
10. Rahal A, Kumar A, Singh V, Yadav B, Tiwari R, Chakraborty S, et al. Oxidative Stress, prooxidants, and antioxidants: The interplay. BioMed Res Int. 2014; 2014: 761264. doi: 10.1155/2014/761264.
11. Furukawa S, Fujita T, Shimabukuro M, Iwaki M, Yamada Y, Nakajima Y, et al. Increased oxidative stress in obesity and its impact on metabolic syndrome. J Clinical Invest. 2004; 114: 1752-61.
12. Roushandeh AM, Salehi IA, Mortazavi M. Protective effects of restricted diet and antioxidants on testis tissue in rats fed with high-fat diet. Iran Biomed J. 2015; 19: 96-101.
13. Chen B, Lam KSL, Wang Y, Wu D, Lam MC, Shen J, et al. Hypoxia dysregulates the production of adiponectin and plasminogen activator inhibitor-1 independent of reactive oxygen species in adipocytes. Biochem Biophys Res Commun.. 2006; 341: 549-56.
14. Higdon JV, Frei B. Obesity and oxidative stress: A direct link to CVD? Arterioscler Thromb Vasc Biol. 2003; 23: 365-7.
15. Abdallah EM. Antibacterial activity of Hibiscus sabdariffa L. calyces against hospital isolates of multidrug resistant Acinetobacter baumannii. J Acute Dis. 2016; 5: 512-6.
16. Usoh IF, Akpan EJ, Etim EO, & Farombi EO. Antioxidant actions of dried flower extracts of Hibiscus sabdariffa L. on sodium arsenite-induced oxidative stress in rats. Pak J Nutr. 2005; 4: 135-41.
17. Mozaffari-Khosravi H, Jalali-Khanabadi BA, Afkhami-Ardekani M, Fatehi F. Effects of sour tea (Hibiscus sabdariffa) on lipid profile and lipoproteins in patients with type II diabetes. J Altern Complement Med. 2009; 15: 899-903.
18. Janson B, Tunsophon S. Protective effect of roselle (Hisbiscus subdariffa L.) on alpha-glucosidase activity and pancreatic function in obese rats with insulin resistance. MSU journal. 2018; Special issue (14th Mahasarakham University Research Conference): 518-28.
19. Tsuda T, Horio F, Uchida K, Aoki H, Osawa T. Dietary cyanidin 3-O-β-D-glucoside-rich purple corn

color prevents obesity and ameliorates hyperglycemia in mice. J Nutr. 2003; 133: 2125-30.
20. Wu HY, Yang KM, Chiang PY. Roselle anthocyanins: Antioxidant properties and stability to heat and pH. Molecules. 2018; 23: 1357. doi: 10.3390/molecules23061357.
21. Obouayeba AP, Djyh NB, Diabate S, Djaman J, Jean David Ng, Koné M, et al. Phytochemical and antioxidant activity of Roselle (Hibiscus sabdariffa L.) petal extracts. Res J Pharm Bio Chem Sci. 2014; 5: 1453-65.
22. Sheba A, Ilakkia A. Anti-obesity effect of Hibiscus sabdariffa L. - A review. Int J Pharm Bio Sci. 2016; 7: 341-5.
23. Ali BH, Wabel NA, Blunden G. Phytochemical, pharmacological and toxicological aspects of Hibiscus sabdariffa L.: A review. Phytother Res. 2005; 19: 369-75.
24. Rodríguez-Medina IC, Beltrán-Debón R, Molina VM, Alonso-Villaverde C, Joven J, Menéndez JA, et al. Direct characterization of aqueous extract of Hibiscus sabdariffa using HPLC with diode array detection coupled to ESI and ion trap MS. J Sep Sci. 2009; 32: 3441-8.
25. La Vignera S, Condorelli R, Vicari E, D'Agata R, Calogero AE. Diabetes mellitus and sperm parameters. J Androl. 2012; 33: 145-53.
26. Shamsi MB, Kumar R, Bhatt A, Bamezai RN, Kumar R, Gupta NP, et al. Mitochondrial DNA mutations in etiopathogenesis of male infertility. Indian J Urol. 2008; 24: 150-4.
27. Amin A, Hamza AA. Effects of roselle and ginger on cisplatin-induced reproductive toxicity in rats. Asian J Androl. 2006; 8: 607-12.
28. World Health Organization. WHO laboratory manual for the examination and processing of human semen, 5th ed. Geneva, Switzerland: WHO Press; 2010. p. 287
29. Kashou AH, du Plessis SS, Agarwal A. The role of obesity in ROS generation and male infertility. In: Agarwal A, Aitken RJ, Alvarez JG, editors. Studies on Men's Health and Fertility. Totowa, NJ: Humana Press; 2012. p. 571-90.
30. Belloc S, Benkhalifa M, Cohen-Bacrie M, Dalleac A, Chahine H, Amar E, et al. Which isolated sperm abnormality is most related to sperm DNA damage in men presenting for infertility evaluation. J Assist Reprod Genet. 2014; 31: 527-32.
31. Zumoff B. Hormonal abnormalities in obesity. Acta Med Scand Suppl. 1988; 723: 153-60.
32. Meng Q, Ren A, Zhang L, Liu J, Li Z, Yang Y, et al. Incidence of infertility and risk factors of impaired fecundity among newly married couples in a Chinese population. Reprod Biomed Online. 2015; 30: 92-100.
33. Ghanayem BI, Bai R, Kissling GE, Travlos G, Hoffler U. Diet-induced obesity in male mice is associated with reduced fertility and potentiation of acrylamide-induced reproductive toxicity. Biol Reprod. 2010; 82: 96-104.
34. Dandona P, Aljada A, Chaudhuri A, Mohanty P, Garg R. Metabolic syndrome: A comprehensive perspective based on interactions between obesity, diabetes, and inflammation. Circulation. 2005; 111: 1448-54.
35. Fraczek M, Kurpisz M. Inflammatory mediators exert toxic effects of oxidative stress on human spermatozoa. J Androl. 2007; 28: 325-33.
36. Mazza G. Anthocyanins in grapes and grape products. Crit Rev Food Sci Nutr. 1995; 35: 341-71.
37. Idris MH, Budin S, Osman M, Mohamed J. Protective role of Hibiscus sabdariffa calyx extract against streptozotocin induced sperm damage in diabetic rats. EXCLI J. 2012; 11: 659-69.
38. Zumoff B, Miller LK, Strain GW. Reversal of the hypogonadotropic hypogonadism of obese men by administration of the aromatase inhibitor testolactone. Metabolism. 2003; 52: 1126-8.
39. Huang T-W, Chang C-L, Kao E-S, Lin J-H. Effect of Hibiscus sabdariffa extract on high fat diet-induced obesity and liver damage in hamsters. Food Nutr Res. 2015; 59: 29018. doi: 10.3402/fnr.v59.29018.