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ANTIOXIDANT PROTECTIVE EFFECT OF TRIHONEY AGAINST HYPERCHOLESTEROLEMIA-INDUCED TESTICULAR OXIDATIVE STRESS IN RABBIT MODEL

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ABSTRACT

Although testicular tissue is highly dependent on oxygen to drive normal spermatogenesis, it is highly sensitive to the toxic effect of reactive oxygen species. On the other hand, honey is a natural product known by its antioxidant properties. Hence, this study aims to investigate the antioxidant effects of Trihoney on testicular tissue of hypercholesterolemic rabbits and compare its effects with atorvastatin. Thirty-six male New Zealand white rabbits were randomly assigned into 6 groups (n=6). Two groups were fed with commercial rabbit pellet and 0 and 0.6 g/kg/day of Trihoney; while, the other four groups were fed with 1% cholesterol diet and 0, 0.3, 0.6 g/kg/day of Trihoney and 2 mg/kg/day of atorvastatin respectively. After 12 weeks, the rabbits were euthanized. The testes were excised and homogenized for measurement of malondialdehyde (MDA) and antioxidant enzymes; superoxide dismutase (SOD) and glutathione peroxidase (GPx). Administration of 1% cholesterol diet increased testicular MDA (p<0.05), reduced SOD (p<0.05) and GPx (p<0.05) activities. Trihoney supplementation and atorvastatin treatment reduced testicular MDA and enhanced testicular SOD activity. There was no detectable effect of Trihoney on testicular GPx activity; meanwhile, treatment with atorvastatin increased GPx activity. Based on the findings of this study, Trihoney and atorvastatin play favourable roles in the reduction of testicular oxidative stress induced by hypercholesterolemia in rabbit model.

KEYWORDS: Hypercholesterolemia; Oxidative stress; Testes; Trihoney; Atorvastatin.

INTRODUCTION

Oxidative stress is induced by the outbalance between production of reactive oxygen species (ROS) and the antioxidant carrying capacity of the affected tissue with subsequent tissue damage.[1] It causes tissue damage through different mechanisms including lipid peroxidation, protein and DNA damage, oxidation of important enzymes and stimulation of pro-inflammatory cytokines.[2] To date, oxidative stress is considered as a major contributory factor in sperm dysfunction and male infertility.[3] It is an acknowledged risk factor for disruption of spermatogenesis at testicular, epididymal and seminal levels.[4] Optimal sperm function depends on the presence of low levels of ROS but high levels lead to lipid peroxidation of sperm membrane, induce DNA damage, aggravate apoptosis, reduce sperm motility, impair sperm function and cause infertility.[5] At the level of testes, the exposure to oxidative stress leads to apoptosis and necrosis of testicular cells and testicular atrophy with subsequent impairment of spermatogenesis causing reduction in sperm count, percentage of normal sperm and sperm motility.[6,7] It also disrupts Leydig cells steroidogenic capacity and ability of germinal epithelium to produce normal sperm.[8] Oxidative stress-induced sperm damage contributes in 30-80% of male factor infertility.[3]

Induction of hyperlipidaemia through a high-energy diet is associated with systemic and testicular oxidative stress.[9,10] High energy diet-induced oxidative stress in animal model resulted in a reduction of sperm concentration, motility and viability. It also resulted in a reduction of testicular and epididymal weights.[11]

On the other hand, honey is a known natural product that has been used by all generations, traditions, and civilizations as a medicine to treat a wide range of diseases.[12] It is known by its antioxidant properties due to the presence of certain components that have antioxidant activities. These components include phenolic acids, flavonoids, glucose oxidase, catalase, ascorbic acids, proteins and carotenoids.[13] Trihoney is a product developed by the Department of Nutrition Sciences, Kulliyyah of Allied Health Sciences, International Islamic University Malaysia (IIUM). It is a mixture of Trigona, Mellifera and Tualang honeys at a specific ratio optimized using Response Surface Methodology (RSM) of Design Expert Version 6.0 software. It is characterized by its high total phenolic content and antioxidant capacity. Trihoney showed its effectiveness in reducing oxidative stress systemically and in atherosclerotic aorta.[14] Therefore, the prime goal of the current study is to evaluate the

antioxidant protective effect of Trihoney against testicular oxidative stress in hypercholesterolemic male rabbits and compare its effect with atorvastatin, which is the most commonly used lipid lowering agent with antioxidant characteristics.[15,16]

MATERIALS AND METHODS

Animals

A total of thirty-six male New Zealand white rabbits of 5-months age were used in the present study. Their environment was maintained under controlled conditions of humidity, temperature and lightning (45-65% humidity, 15-21°C temperature with 12hours light/dark cycles). The rabbits were individually housed in stainless-steel cages designed for rabbits (Tecniplast, Italy), and they had free access to water and food. The details of the current animal experiment were reviewed and approved by the International Islamic University Malaysia Institutional Animal Care and Use Committee (IACUC-IIUM), IIUM, Kuantan campus.

Preparation of 1% cholesterol diet

Ten grams of pure cholesterol powder (Nacalai-Tesque, Japan) were emulsified in 20 mL (2%) of cholesterol-free coconut oil (Certified Organic, Philippines). Then, the emulsion was poured onto 970 grams of rabbit pellet and mixed thoroughly. The high cholesterol diet was kept at room temperature. It was repeatedly and freshly prepared twice weekly.[17]

Trihoney and Atorvastatin doses

Two different doses of Trihoney (0.6 g/kg/day and 0.3 g/kg/day) were used in this experiment. The animal equivalent dose of Trihoney was calculated based on the conversion of human equivalent dose to animal equivalent dose and administered orally. Atorvastatin tablets (Prague-Czech) were crushed into fine powder, reconstituted in 1 mL of distilled water and given orally at a dose of 2 mg/kg/day.[18,19]

Experimental design

All rabbits were fed with commercial rabbit pellet for 2 weeks to allow acclimatization before the commencement of the experiment. Thereafter, the rabbits were randomly assigned into 6 groups of six animals each and received different diets as follows:

Group 1: Control (C) (Commercial rabbit pellet).

Group 2: CH (Commercial rabbit pellet with a daily oral dose of 0.6 g/kg/day Trihoney).

Group 3: HCD (1% cholesterol diet)

Group 4: DAt (1% cholesterol diet with a daily oral dose of 2 mg/kg/day atorvastatin).
Group 5: DH1 (1% cholesterol diet with a daily oral dose of 0.3 g/kg/day Trihoney).
Group 6: DH2 (1% cholesterol diet with a daily oral dose of 0.6 g/kg/day Trihoney).

Measurements of body weight were performed and recorded once weekly and Trihoney and atorvastatin doses were adjusted accordingly. The experiment was continued for 12 weeks. The aim of having CH group was to determine the effect of Trihoney on oxidative stress parameters of normocholesterolemic rabbits.

Animal sacrificing and testes harvesting

At the end of the 12 weeks, the rabbits were fasted overnight and anaesthetized through intramuscularly injected ketamine/ xylazine combination at a dose of 50/10 mg/kg body weight.[20] Under complete clean conditions, ventral midline incision was made through the abdominal cavity and pelvis to expose the internal organs. The left testis was allowed to be perfused with normal saline to clear it from the blood.[21] Systemic perfusion was performed using iced-cold normal saline through left ventricular approach. The left testis from each rabbit was excised, cut into pieces and preserved at −80°C for homogenate preparation.[22]

Preparation of testicular tissue homogenate

Testicular tissue of each rabbit was weighed and homogenized to make 10% homogenate (w/v)[23] in ice-cold phosphate buffer saline[24] using Bullet Blender blue homogenizer (Next Advance, USA). Twenty microliters of protease inhibitor cocktail (Nacalai tesque, Japan) was added to each 1 mL of phosphate buffer saline. The homogenate was then centrifuged in a refrigerated centrifuge (ThermoFisher Scientific, Germany) at 10000 g for 15 min at 4 °C. The obtained supernatant was stored at −80°C and used as a sample for assaying of testicular MDA and the antioxidant enzymes.[23]

Protein assay in testicular homogenate

Protein concentration in the homogenate was measured using protein assay CBB solution assay kit (Ready to Use) from (Nacalai Tesque, Japan) according to the manufacturer's protocol. Albumin bovine serum (Nacalai Tesque, Japan) was used as a standard.[23]

Analysis of malondialdehyde and antioxidant enzymes

The levels of intra-testicular MDA (Cell Biolabs, USA), superoxide dismutase (Cell Biolabs, Inc. USA) and glutathione peroxidase (Abnova, Taiwan) were estimated

spectrophotometrically using assay kits. The analysis was performed according to the manufacturer's instructions.

Statistical analysis

Statistical Package for the Social Sciences Version 21 (SPSS Inc., Chicago, Illinois, USA) program was used for data processing. Data were expressed as means and standard deviations. One-way analysis of variance test (ANOVA) was used for data analysis followed by a *post hoc* test to determine any significant differences between the means of the independent groups. Differences were considered statistically significant at *p* values less than 0.05.

RESULTS

The results of testicular MDA, SOD and GPx after 12 weeks of 1% cholesterol diet, Trihoney and atorvastatin administration are given in Table 1. There was no significant difference between CH group and the control in testicular levels of MDA, SOD and GPx. Administration of 1% cholesterol diet increased testicular MDA level in the high cholesterol diet group which expressed significantly higher level than the control ($p<0.05$). The testicular MDA level was lower in atorvastatin and Trihoney received groups than the high cholesterol diet group; however, the significant difference was found only between the atorvastatin treated group and the high cholesterol diet group ($p<0.05$). There was no significant difference neither between Trihoney received groups and the control group ($p>0.05$) nor between the atorvastatin treated group and the control group in the testicular MDA level ($p>0.05$). Feeding of rabbits with 1% cholesterol diet significantly reduced testicular SOD activity in the high cholesterol diet group when compared to the control ($p<0.05$). Administration of Trihoney or treatment with atorvastatin enhanced the enzyme activity in their respective groups, which showed no significant difference from the control ($p>0.05$). Superoxide dismutase activity was comparable in Trihoney and atorvastatin received groups. As per testicular GPx activity, the high cholesterol diet group and the Trihoney received groups exhibited a significant reduction compared to the control ($p<0.05$). There was no significant difference neither between the high cholesterol diet group and the Trihoney received groups ($p>0.05$) nor between the high cholesterol diet group and the atorvastatin treated group ($p>0.05$). Although the activity of GPx was lower in the atorvastatin treated group than the control, the atorvastatin treated group showed no significant difference from the control ($p>0.05$).

Table 1: Effects of 1% Cholesterol Diet, Trihoney and Atorvastatin on Testicular Malondialdehyde, Superoxide Dismutase and Glutathione Peroxidase.

Parameter Group	MDA (µg/mg protein)	SOD (%)	GPx (mU/ mg protein)
C	0.13 ± 0.07^{a}	28.52 ± 7.67^{a}	79.71 ± 18.28^{a}
CH	0.12 ± 0.05^{a}	27.79 ± 2.21^{a}	73.71 ± 6.39^{a}
HCD	0.29 ± 0.13^{b}	17.15 ± 5.37^{b}	45.35 ± 13.38^{b}
DAt	$0.13\pm0.08^{a,c}$	$25.26\pm9.92^{a,b}$	$66.66\pm47.29^{a,b}$
DH1	$0.22\pm0.10^{a,b,c}$	$21.10\pm0.92^{a,b}$	45.55 ± 5.86^{b}
DH2	$0.19\pm0.16^{a,b,c}$	$22.15\pm5.78^{a,b}$	41.32 ± 1.97^{b}

Data were analysed using one-way analysis of variance (ANOVA). A Post Hoc comparison using *LSD* test was used to test the significant difference between the groups. Values are given as mean ± standard deviation of mean. ^{a,b,c} Values not sharing a common superscript letter within the same column differ significantly at $p<0.05$. C: commercial pellet; CH: commercial pellet plus 0.6 g/kg/day Trihoney; HCD: 1% cholesterol diet; DH1: 1% cholesterol diet plus 0.3 g/kg/day Trihoney; DH2: 1% cholesterol diet plus 0.6 g/kg/day Trihoney; DAt: 1% cholesterol diet plus 2 mg/kg/day atorvastatin; MDA: malondialdehyde; SOD: superoxide dismutase; GPx: glutathione peroxidase.

DISCUSSION

In the current study, rabbits fed with 1% cholesterol diet experienced a significant increase in testicular MDA accompanied by a significant reduction in testicular SOD and GPx activities. These findings indicate that the rabbits from this group were exposed to testicular oxidative stress. Trihoney supplementation reduced MDA level; meanwhile, enhanced testicular SOD activity with no detectable effect on testicular GPx activity. Treatment of rabbits with atorvastatin reduced testicular MDA and enhanced testicular SOD and GPx activities.

Malondialdehyde is the end product of lipid peroxidation and commonly used marker to evaluate lipid peroxidation of serum, plasma and tissues.[25,26] Enzymatic antioxidants are termed as natural antioxidants, they prevent oxidative stress-induced cellular damage *via* scavenging excessively produced ROS.[27] They consist of superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx) and glutathione reductase (GR).[28]

In several previous studies hypercholesterolemia has been linked to lipid peroxidation and shift of the pro-oxidant-antioxidant balance toward the pro-oxidation in serum and different tissues.[29] It induced oxidative stress in serum and aorta,[14] in plasma and liver[30] and in

liver, brain, heart, aorta and platelets.[31] The increase in the oxidative stress has been positively associated with the duration of hypercholesterolemia.[32] Hypercholesterolemia-induced oxidative stress has been demonstrated in different animal models as rabbits,[14] rats[29] and transgenic mice.[33] In human, it was observed that MDA level is higher; whereas, GPx and SOD are lower in hypercholesterolemic patients than the healthy subjects.[34] The disturbance in pro-oxidant-antioxidant balance was also reported to be associated with the increase in total cholesterol (TC) and low density lipoprotein (LDL-c) in the healthy individuals.[35] As far as testicular tissue is concerned, feeding of rats with a high-energy diet induced testicular oxidative stress evidenced by an increase in testicular MDA and a reduction in testicular SOD activity.[10]

Hypercholesterolemia induces oxidative stress either through increase production of free radicals or reduction of the antioxidant reserve.[32] Although testicular tissue is highly dependent on oxygen to drive normal spermatogenesis, it is highly sensitive to the toxic effects of ROS.[8] In hyperlipidaemic animals, testicular oxidative stress is due to mitochondrial dysfunction as a result to testicular damage, which caused by hyperlipidaemia.[36] Dysfunction of the mitochondria induces more ROS production that produces further damage to the mitochondrial membrane followed by more ROS production.[5]

Supplementation of Trihoney reduced testicular MDA and enhanced testicular SOD activity as compared to the administration of 1% cholesterol diet alone. These improvements demonstrate that Trihoney has the potential to protect against testicular oxidative stress in hypercholesterolemic rabbits. The presence of some evidence of oxidative stress in Trihoney received groups is explained by the continuation of the high-cholesterol diet administration, which is considered to be an ongoing source of oxidative stress. It is well-known that honey both from stingless bees and from honey bees has a good antioxidant capacity.[37] The antioxidant properties of honey are attributed to its constituents such as glucose oxidase, catalase, ascorbic acid, flavonoids, phenolic acids, carotenoid derivatives, organic acids, Maillard reaction products, amino acids and proteins.[38] Enhancement of testicular SOD *via* Trihoney might be due to the increase of SOD bioavailibity through reduction of testicular cells damage by Trihoney supplementation.[23] This protective effect might explain the improvements of testicular and epididymal histopathological changes with improvement of reproductive hormones and sperm parameters in Trihoney received rabbits in our previously

published studies.[17,39–41] Superoxide dismutase is the main cellular antioxidant enzyme in all types of cells. It is of high importance in testicular tissue which contain, in addition to cytosolic and mitochondrial SOD, an unusual extracellular SOD produced by Sertoli and germ cells.[8,42] Superoxide dismutase prevents lipid peroxidation of plasma membrane by neutralizing intracellular and extracellular superoxide anion. It converts superoxide radicals to hydrogen peroxide and oxygen. Although GPx is considered to be stable enzyme, it may be suppressed in severe oxidative stress and in chronically high levels of H_2O_2.[43,44] This could explain the suppression of GPx in Trihoney received rabbits in the present study.

In line with the current study, administration of honey to rats exposed to cigarette smoke reduced testicular oxidative stress marker and restored the antioxidant defence system including SOD, catalase and GPx.[23] To further support the ability of honey to enhance the antioxidant defence system of the body, consumption of honey supplemented-diet increased blood antioxidant agents such as vitamin C, beta-carotene, uric acid and glutathione reductase in healthy individuals.[45] Moreover, consumption of honey by healthy adults was capable to raise plasma total phenolic contents, plasma antioxidants and reducing capacities.[46] The antioxidant activity of Trihoney is probably due to its high total phenolic content and high free radical scavenging activity. Trihoney is rich in phenolic components such as Quercetin, aempferol, rutin, cathecin, maleic acid, caffeic acid, cinnamic acid, gallic acid, p-hydroxybenzoic acid, salicylic acid, sinapic acid and vanillic acid, which characterized by their antioxidant activities.[47] Among these identified phenolic components, sinapic acid and cinnamic acid showed high association with antioxidant activities.[48] Quercetin, Catechin and rutin are strong radical scavengers and inhibitors of lipid peroxidation.[49]

In the present study, treatment of rabbits with atorvastatin reduced testicular MDA and enhanced testicular SOD and GPx activities. It is well-established that statins have a wide spectrum of effects beyond cholesterol lowering activities. These effects so-called the pleiotropic effects, from which the antioxidant effect is an important property.[34] The antioxidant effect of atorvastatin was demonstrated in different tissues and in different models.[50–52] It was also speculated that the antioxidant effect of atorvastatin was mediated by activation of nuclear factor erythroid 2-related factor 2 (Nrf2) signalling pathway that has a key role in stimulation of endogenous antioxidant enzymes.[15] The antioxidant activity was suggested as one of the earliest beneficial effects of atorvastatin even before its hypolipidaemic effect.[16] Even though atorvastatin showed an improvement in testicular

antioxidant enzymes and reduced testicular MDA, it showed the worst effects on the testicular histology, serum and intra-testicular testosterone and sperm analysis in our recently published studies.[17,39,40] This may indicate that atorvastatin itself has a toxic effect on testicular tissue through a mechanism other than the oxidative stress mechanism.

CONCLUSION

Trihoney showed its effectiveness as a potential protective natural product that minimizes testicular oxidative stress in hypercholesterolemic rabbit model, through reduction of testicular MDA and enhancement of testicular SOD activity. The antioxidant activity of Trihoney is probably due to its high total phenolic content and high free radical scavenging activity. This antioxidant activity likely to be one of the main underlying mechanisms through which Trihoney improved testicular and epididymal histopathological changes with a subsequent improvement of reproductive hormones and sperm parameters. Atorvastatin reduced testicular oxidative stress; however, it has been reported to have a negative impact on testicular and epididymal tissues, male reproductive hormones and sperm parameters, which indicates that atorvastatin itself may have a toxic effect on male reproductive system *via* a mechanism other than the oxidative stress damage.

REFERENCES

1. Hamad Zubi ZB, Hamad Alfarisi HA. Hyperlipidemia and male infertility. Egypt J Basic Appl Sci, 2021; 1, 8(1): 385–96.
2. Katakwar P, Metgud R, Naik S, Mittal R. Oxidative stress marker in oral cancer: A review. J Cancer Res Ther, 2016; 12(2): 438–46.
3. Bisht S, Faiq M, Tolahunase M, Dada R. Oxidative stress and male infertility. Nat Rev Urol, 2017; 14(8): 470–85.
4. Sarkar O, Bahrainwala J, Sambamurthy C sekaran, Kotari S, Mathur PP, Agarwal A. Impact of inflammation on male fertility. Front Biosci, 2011; E3(1): 89–95.
5. Agarwal A, Majzoub A. Free Radicals in Andrology. In: Balercia G, Gandini L, Lenzi A, Lombardo F, editors. Trends in Andrology and Sexual Medicine. Cham: Springer International Publishing, 2017; 1–21. (Trends in Andrology and Sexual Medicine; vol. 1).
6. Aziz N, Saleh RA, Sharma RK, Lewis-Jones I, Esfandiari N, Thomas AJ, et al. Novel association between sperm reactive oxygen species production, sperm morphological defects, and the sperm deformity index. Fertil Steril, 2004; 2(81): 349–54.
7. Sanaz A, Amirreza T, Solmaz R, Keyvan A, Elham Y. Cadmium and male infertility. J

Infertil Reprod Biol, 2014; 2(2): 62–9.

8. Aitken RJ, Roman SD. Antioxidant Systems and Oxidative Stress in the Testes. Oxid Med Cell Longev, 2008; 1(1): 15–24.
9. Han J-M, Lee J-S, Kim H-G, Seol I-C, Im H-J, Cho J-H, et al. Synergistic effects of Artemisia iwayomogi and Curcuma longa radix on high-fat diet-induced hyperlipidemia in a mouse model. J Ethnopharmacol, 2015; 173: 217–24.
10. Galaly SR, Hozayen WG, Amin KA, Ramadan SM. Effects of Orlistat and herbal mixture extract on brain, testes functions and oxidative stress biomarkers in a rat model of high fat diet. Beni-Suef Univ J Basic Appl Sci, 2014; 3(2): 93–105.
11. Chen X, Gong L, Xu J. Probiotics Attenuate Sperm Damage Induced by Oxidative Stress in Rats. Int Conf Biomed Eng Biotechnol, 2012; 314: 446–8.
12. Israili ZH. Antimicrobial Properties of Honey. Am J Ther, 2014; 21(4): 304–23.
13. Alvarez-Suarez JM, Tulipani S, Díaz D, Estevez Y, Romandini S, Giampieri F, et al. Antioxidant and antimicrobial capacity of several monofloral Cuban honeys and their correlation with color, polyphenol content and other chemical compounds. Food Chem Toxicol, 2010; 48: 2490–9.
14. Alfarisi HAH, Ibrahim M, Mohamed ZBH, Hamdan AH, Che Mohamad CA. Trihoney reduces lipid peroxidation and enhances antioxidant enzyme activities in hypercholesterolaemic atherosclerotic rabbits. Int Food Res J., 2020; 27(3): 568–75.
15. Sun G, Li Y, Ji Z. Atorvastatin attenuates inflammation and oxidative stress induced by ischemia/reperfusion in rat heart via the Nrf2 transcription factor. Int J Clin Exp Med., 2015; 8(9): 14837–45.
16. Marketou ME, Zacharis EA, Nikitovic D, Ganotakis ES, Parthenakis FI, Maliaraki N, et al. Early effects of simvastatin versus atorvastatin on oxidative stress and proinflammatory cytokines in hyperlipidemic subjects. Angiology, 2006; 57(2): 211–8.
17. Mohamed ZBH, Hamad Alfarisi HA, Abdul Wahab AY, Abd Fuaat A, Che Mohamad CA, Ibrahim M. Trihoney improves testicular weight change and histopathological alterations in hypercholesterolemic rabbits. Asia Pacific J Mol Biol Biotechnol, 2020; 28(3): 75–87.
18. Reagan-Shaw S, Nihal M, Ahmad N. Dose translation from animal to human studies revisited. FASEB J., 2007; 22(3): 659–61.
19. Du B, Xu G, Cao H, Cui W, Lin S, Liu Y, et al. Effects of atorvastatin on expression of ICAM-1 in atherosclerotic rabbits. J Cardiovasc Med, 2013; 14(2): 120–6.
20. Hamad Alfarisi HAS, Ibrahim M, Hamad Mohamamed Z b., Hamdan AH, Che Mohamad

CA. Trihoney Suppresses Soluble Adhesion Molecules (ICAM-1 and VCAM-1 in Hypercholesterolemic Atherosclerotic Rabbits: A Comparative Study with Atorvastatin. Sains Malaysiana, 2020; 30, 49(6): 1313–22.

21. Graham J. Homogenization of Mammalian Tissues. Sci World J., 2002; 2: 1626–9.
22. Ilbey YO, Ozbek E, Simsek A, Cekmen M, Otunctemur A, Somay A. Chemoprotective Effect of a Nuclear Factor- B Inhibitor, Pyrrolidine Dithiocarbamate, Against Cisplatin-Induced Testicular Damage in Rats. J Androl, 2009; 1, 30(5): 505–14.
23. Mohamed M, Sulaiman SA, Jaafar H, Sirajudeen KNS. Antioxidant Protective Effect of Honey in Cigarette Smoke-Induced Testicular Damage in Rats. Int J Mol Sci, 2011; 29, 12(9): 5508–21.
24. Wagner IV, Klöting N, Atanassova N, Savchuk I, Spröte C, Kiess W, et al. Prepubertal onset of obesity negatively impacts on testicular steroidogenesis in rats. Mol Cell Endocrinol, 2016; 437: 154–62.
25. Mikail MA, Ahmed IA, Ibrahim M, Hazali N, Abdul Rasad MSB, Abdul Ghani R, et al. Baccaurea angulata fruit inhibits lipid peroxidation and induces the increase in antioxidant enzyme activities. Eur J Nutr, 2016; 20, 55(4): 1435–44.
26. Atilgan D, Parlaktas BS, NihatUluocak, Erdemir F, Kilic S, Erkorkmaz U, et al. Weight Loss and Melatonin Reduce Obesity-Induced Oxidative Damage in Rat Testis. Adv Urol, 2013; 36(3): 1–6.
27. Agarwal A, Gupta S, Sharma RK. Role of oxidative stress in female reproduction. Reprod Biol Endocrinol, 2005; 3(28): 1–21.
28. Bansal AK, Bilaspuri GS. Impacts of Oxidative Stress and Antioxidants on Semen Functions. Vet Med Int, 2011; 2011: 1–7.
29. Küçükgergin C, Aydın AF, Özdemirler-Erata G, Mehmetçik G, Koçak-Toker N, Uysal M. Effect of Artichoke Leaf Extract on Hepatic and Cardiac Oxidative Stress in Rats Fed on High Cholesterol Diet. Biol Trace Elem Res, 2010; 4, 135(1–3): 264–74.
30. Mahfouz MM, Kummerow FA. Cholesterol-rich diets have different effects on lipid peroxidation, cholesterol oxides, and antioxidant enzymes in rats and rabbits. J Nutr Biochem, 2000; 11(5): 293–302.
31. De La Cruz JP, Quintero L, Villalobos MA, Sánchez de la Cuesta F. Lipid peroxidation and glutathione system in hyperlipemic rabbits: influence of olive oil administration. Biochim Biophys Acta - Mol Cell Biol Lipids, 2000; 1485(1): 36–44.
32. Prasad K, McNair ED, Qureshi AM, Casper-Bell G. Vitamin E slows the progression of hypercholesterolemia-induced oxidative stress in heart, liver and kidney. Mol Cell

Biochem, 2012; 20, 368(1–2): 181–7.

33. Csont T, Bereczki E, Bencsik P, Fodor G, Görbe A, Zvara Á, et al. Hypercholesterolemia increases myocardial oxidative and nitrosative stress thereby leading to cardiac dysfunction in apoB-100 transgenic mice. Cardiovasc Res, 2007; 1, 76(1): 100–9.
34. Yilmaz MI, Kocar IH, Baykal Y, Kilic M, Sonmez A, Bulucu F, et al. Effects of statins on oxidative stress. Biol Trace Elem Res, 2004; 98(2): 119–27.
35. Balkan J, Dogru-Abbasoglu S, Aykac-Toker G, Uysal M. Serum pro-oxidant-antioxidant balance and low-density lipoprotein oxidation in healthy subjects with different cholesterol levels. Clin Exp Med, 2004; 1, 3(4): 237–42.
36. Zhang K, Lv Z, Jia X, Huang D. Melatonin prevents testicular damage in hyperlipidaemic mice. Andrologia, 2012, 44(4): 230–6.
37. Rao PV, Krishnan KT, Salleh N, Gan SH. Biological and therapeutic effects of honey produced by honey bees and stingless bees: a comparative review. Rev Bras Farmacogn, 2016; 26(5): 657–64.
38. Bogdanov S, Jurendic T, Sieber R, Gallmann P. Honey for nutrition and health: A review. J Am Coll Nutr, 2008; 27(6): 677–89.
39. Mohamed ZBH, Ibrahim M Bin, Alfarisi HAH, Abdul Wahab AY, Abd Fuaat AB, Che Mohamad CA. Honey Improves Sperm Parameters in High Cholesterol Diet-Fed Male Rabbits. ASM Sci J., 2021; 17, 15: 1–10.
40. Hamad Mohamed Z, Brahim M, Hamad Alfarisi H, Abdul Wahab A, Abd Fuaat A, Che Mohamad C. Effect of Trihoney (A Mixture of Trigona, Mellifera and Tualang) on Male Reproductive Hormones and Insulin Resistance in Hypercholesterolaemic Rabbits. IMJM, 2020; 19(3): 21–9.
41. Mohamed ZBH, Ibrahim M, Alfarisi HAH, Abdul Wahab AY, Abd Fuaat A, Mohamad CAC. Trihoney ameliorates hypercholesterolemia-induced epididymal histopathological changes in male rabbits. AsPac J Mol Biol Biotechnol, 2020; 28(1): 90–100.
42. Stapleton PA, Goodwill AG, James ME, Brock RW, Frisbee JC. Hypercholesterolemia and microvascular dysfunction : Interventional Strategies. J Inflamm, 2010; 7(54): 1–10.
43. Dworzański J, Strycharz-Dudziak M, Kliszczewska E, Kiełczykowska M, Dworzańska A, Drop B, et al. Glutathione peroxidase (GPx) and superoxide dismutase (SOD) activity in patients with diabetes mellitus type 2 infected with Epstein-Barr virus. PLoS One, 2020; 15(3): 1–10.
44. Ryan MJ, Dudash HJ, Docherty M, Geronilla KB, Baker BA, Haff GG, et al. Aging-Dependent Regulation of Antioxidant Enzymes and Redox Status in Chronically Loaded

Rat Dorsiflexor Muscles. J Gerontol A Biol Sci Med Sci, 2008, 2008; 63(10): 1015–26.

45. Al-Waili NS. Effects of Daily Consumption of Honey Solution on Hematological Indices and Blood Levels of Minerals and Enzymes in Normal Individuals. J Med Food, 2003; 6(2): 135–40.
46. Schramm DD, Karim M, Schrader HR, Holt RR, Cardetti M, Keen CL. Honey with high levels of antioxidants can provide protection to healthy human subjects. J Agric Food Chem, 2003; 51(6): 1732–5.
47. Ly C, Yockell-Lelièvre J, Ferraro ZM, Arnason JT, Ferrier J, Gruslin A. The effects of dietary polyphenols on reproductive health and early development. Hum Reprod Update, 2015; 21(2): 228–48.
48. Khang D, Dung T, Elzaawely A, Xuan T. Phenolic Profiles and Antioxidant Activity of Germinated Legumes. Foods, 2016; 5(27): 1–10.
49. Kumar S, Pandey AK. Chemistry and Biological Activities of Flavonoids: An Overview. Sci World J., 2013; 2013(4): 1–16.
50. Aktay G, Gürsoy ŞÖ, Uyumlu U, Ünüvar S, İlhan N. Protective effect of atorvastatin on oxidative stress in streptozotocin-induced diabetic rats independently their lipid-lowering effects. J Biochem Mol Toxicol, 2019; 33(5): 1–6.
51. Jorge PAR, Almeida EA De, Ozaki MR, Jorge M, Carneiro A. Effects of atorvastatin, fluvastatin, pravastatin, and simvastatin on endothelial function, lipid peroxidation, and aortic atherosclerosis in hypercholesterolemic rabbits. Arq Bras Cardiol, 2005; 84(4): 314–9.
52. Bolayirli IM, Aslan M, Balci H, Altug T, Hacibekiroglu M, Seven A. Effects of atorvastatin therapy on hypercholesterolemic rabbits with respect to oxidative stress, nitric oxide pathway and homocysteine. Life Sci, 2007; 81(2): 121–7.