

Honey Improves Sperm Parameters in High Cholesterol Diet-Fed Male Rabbits

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Overconsumption of a high-energy diet has a negative impact on sperm motility, morphology, vitality and concentration. Hence, this study aims to investigate the effects of honey on sperm parameters of high cholesterol diet-fed rabbits and compare its effects with atorvastatin. Forty-eight male New Zealand white rabbits were assigned into 6 groups: Control (C): commercial pellet; CH: commercial pellet and 600 mg/kg/day Trihoney; HCD: 1% cholesterol diet; DH1: 1% cholesterol diet and 300 mg/kg/day Trihoney; DH2: 1% cholesterol diet and 600 mg/kg/day Trihoney; DAT: 1% cholesterol diet and 2 mg/kg/day atorvastatin. After 12 weeks, each rabbit was anaesthetized. Sperm were obtained from the cauda of the epididymis. Percentages of progressive (PM) and total motility (TM) were assessed using light microscope. Vitality and morphology were assessed using Eosin-Nigrosin stain. Sperm concentration was calculated using haemocytometer. Administration of 1% cholesterol diet reduced the percentages of PM, TM and normal sperm. Treatment with atorvastatin reduced the percentages of PM, TM, live and normal sperm. A marked reduction in sperm concentration was detected in the HCD and DAT groups. Trihoney groups expressed significantly higher percentages of PM, TM, normal sperm, live sperm and sperm concentration than the HCD and DAT groups. These results indicate that Trihoney has the potential to minimize the negative impacts of a high cholesterol diet on sperm parameters.

Keywords: high cholesterol diet; sperm parameters; Trihoney; atorvastatin

I. INTRODUCTION

Spermatogenesis is a multistep process through which sperm are produced from male germ cells in the seminiferous tubules of the testes. It is a sensitive process requiring optimal conditions to occur perfectly as it is essential for male reproduction (Azenabor *et al.*, 2015). Poor semen quality is a widespread problem and it is one of the causes of infertility in approximately half the number of couples seeking assistance

in order to achieve parenthood (Jensen *et al.*, 2013). Sperm count, motility and morphology are parameters used to estimate male fertility (Wong *et al.*, 2000). They provide information about the functions of the seminiferous tubules, epididymis, and accessory sex glands. They also give an idea about the diagnosis and prognosis of infertility (Esteves, 2014). Abnormal sperm analysis includes oligozoospermia (sperm count less than 39×10^6 /ejaculate or sperm concentration less than 15×10^6 /mL), asthenozoospermia

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(progressive motility less than 32%) and teratozoospermia (normal morphology less than 4%) or a combination of any of these, in addition to several others (WHO, 2010). Overconsumption of a high energy diet has a negative impact on spermatogenesis at central and gonadal levels. It disrupts energy supply to male germ cells, induces sperm defects and spermatogenesis arrest (Rato *et al.*, 2014). Hypercholesterolemia induced by a high cholesterol diet was found to reduce sperm functionality, count and motility coupled with an increase in sperm morphological abnormalities (Saez Lancellotti *et al.*, 2010, 2013). Furthermore, it impairs spermatogenesis (Mohamed *et al.*, 2020a) and markedly reduces epididymal mature sperm count (Mohamed *et al.*, 2020b). Although extensive research has been conducted and abundant information are available about male infertility, the use of medical treatment is associated with various side effects (Dabaja & Schlegel, 2014) and assisted reproductive technology is expensive and not always available and successful (Mora-Esteves & Shin, 2013). The inclination of the general population nowadays is toward the use of herbal medicine (Zarei *et al.*, 2014). Trihoney is a mixture of three types of honey namely Trigona, Mellifera and Tualang at a specific ratio. It is a product developed by the Department of Nutrition Sciences, Kulliyah of Allied Health Sciences, International Islamic University Malaysia. It was optimized using Response Surface Methodology (RSM) of Design Expert Version 6.0 software. It has a high total phenolic content. Hence, the objective of the current study is to investigate the protective effects of Trihoney on sperm parameters of high cholesterol diet-fed male rabbits and compare its effects with atorvastatin, which is the most commonly used among statins that are the first line lipid lowering agents.

II. MATERIALS AND METHOD

A. Ethics Statement

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.

B. Chemicals

Atorvastatin (Atorvastatin Winthrop®, Prague-Czech), cholesterol powder (Nacalai-Tesque, Japan), cholesterol-free coconut oil (Certified Organic, Philippines), sperm washing media (modified HTF medium with human serum albumin 5.0 mg/mL, Irvine Scientific, USA), VitalScreen kit (FertiPro, Belgium).

C. Animals and Diets

Forty-eight mature male New Zealand white rabbits with body weight of 2000–2500g were used in this study. They were housed in separate cages at a temperature of 15–21°C and humidity of 45–65% with 12hours light/dark cycles. They had free access to water and pellet for an acclimatization period of 2 weeks. The rabbits were then randomly assigned into one of the six groups, with eight animals each as demonstrated in Table 1.

Table 1. Animal grouping of the current experiment

Group	Given name	Diet and Treatment
I	C	Commercial rabbit pellet + No treatment
II	CH	Commercial pellet + 600 mg/kg/day of Trihoney
III	HCD	1 % cholesterol + No treatment
IV	DAt	1% cholesterol +2 mg/kg/day atorvastatin
V	DH1	1 % cholesterol + 300 mg/kg/day of Trihoney
VI	DH2	1 % cholesterol + 600 mg/kg/day of Trihoney

The high cholesterol diet was prepared according to Mohamed *et al.* (2020b). Trihoney doses were calculated according to Reagan-Shaw *et al.* (2007) by conversion of human equivalent dose, which is 0.1– 0.2 g/Kg/day (Mohamed *et al.*, 2011; Natural Medicines Comprehensive Database, 2016), to animal equivalent dose. The used dose of atorvastatin was 2 mg/kg/day (Du *et al.*, 2013). Atorvastatin was reconstituted in 1 mL of distilled water before its oral administration (Song *et al.*, 2014). The duration of

spermatogenesis in rabbits is between 7 to 8 weeks and the studies that aim to determine the effects of exogenous factors on spermatogenesis should be continued at least for 10 weeks (International Rabbit Reproduction Group, 2005), therefore this experiment was carried out for 12 weeks.

The purpose of having CH group was to evaluate the enhancing effect of Trihoney on sperm parameters of normocholesterolemic rabbits. It also investigates the safety of Trihoney to be consumed without negative impacts of Trihoney itself on the tested parameters.

D. Preparation of Caudal Epididymal Sperm Suspension

At the end of the 12 weeks, the rabbits fasted overnight and anaesthetized using intramuscular injection of ketamine/xylazine at a dose of 50/10 mg/kg body weight (Zhao *et al.*, 2013). A longitudinal midline incision was made through the abdominal cavity and pelvis. The right testis and epididymis were immediately and carefully excised and separated from each other. The sperm were obtained from the cauda of the excised right epididymis (Chen *et al.*, 2012; Yamamoto *et al.*, 1999). The cauda was placed immediately into a sterile petri dish (Nalgene, Denmark) containing 4 mL of sperm washing media pre-warmed at 37°C (Yamamoto *et al.*, 1999; Ilbey *et al.*, 2009). The cauda was minced and incubated in the pre-warmed sperm washing media for 5 to 10 minutes to allow recovery and dispersion of sperm (Chen, Gong & Xu, 2012; Rato *et al.*, 2013).

E. Analysis of Sperm Motility

Ten microliters of sperm suspension were placed on a pre-warmed slide (37°C) and covered with a pre-warmed cover slip (37°C) size 22 mm × 22 mm (MENZEL-GLASER®), to provide a chamber with 20 µm depth, and examined under ×400 magnification of the microscope (Leica, Germany) (WHO, 2010). Ten random fields were assessed and the average value was used as an indicator of motility (Rato *et al.*, 2013). The percentages of progressive motility, total motility and immotile sperm were assessed for each rabbit (WHO, 2010).

F. Analysis of Sperm Vitality and Morphology

Sperm vitality was assessed by means of the dye exclusion method using Eosin-Nigrosin staining technique according to the protocol of VitalScreen kit (FertiPro, Belgium). A total of 200 sperm were assessed under ×400 and ×1000 magnifications to determine the percentages of live and dead sperm. Live sperm were identified by their white or light pink heads; whereas, dead sperm have dark pink or red coloured heads (WHO, 2010; Chen, Gong & Xu, 2012). Sperm morphology percentages were assessed from the same smears prepared for vitality analysis with assessment of 200 sperm under ×400 and ×1000 magnifications. The sperm were tested for head defects: large or small, tapered, pyriform, round, amorphous or double heads; neck and midpiece defects: asymmetrical insertion of the midpiece into the head, thick or irregular, bent or abnormally thin; tail defects: coiled, bent, short or double tails or any combination of these (Ping *et al.*, 2014; WHO, 2010).

G. Analysis of Sperm Concentration

Sperm concentration was calculated using haemocytometer (Neubauer-improved, Hirschmann, European Union). An aliquot of 50 µL from the sperm suspension was diluted with distilled water in a sterile Eppendorf tube; two replicates were prepared. The dilution was performed based on the number of sperm which was seen in the preparation used for motility. If the sperm count per ×400 magnification was more than 101, a dilution of 1:20 (50 µL: 950 µL) was used; for sperm count between 16-100, the dilution was 1:5 (50 µL:200 µL); whereas, a dilution of 1:2 (50 µL:50 µL) was used if the sperm count was below 15 (WHO, 2010). After mixing the samples well, 10 µL from each replicate dilution was transferred into one of the two chambers of the haemocytometer which was kept horizontally in a humid chamber for 5 minutes to prevent drying. Two-hundred sperm were assessed in each replicate with assessment of the central grid (number 5) at first; if the count did not reach 200 sperm in the central grid, the counting was continued in the adjacent grids number 4 and number 6. For the samples of low concentrations, the counting was continued in the rest of the grids and involved all nine grids in some rabbits (WHO, 2010). The equation

used for calculation of the concentration is as follows (WHO, 2010):

$$C = (N/n) \times (1/20) \times \text{dilution factor (sperm} \times 10^6/\text{mL)}$$

where: C= Concentration, N= Total number of sperm in both chambers and n= number of rows or grids examined for the replicates.

H. Statistical Analysis

Data were processed using the Statistical Package for the Social Sciences Version 21 (SPSS Inc., Chicago, Illinois, USA). They 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 to be statistically significant at p values less than 0.05.

III. RESULT AND DISCUSSION

The deleterious effects of a high cholesterol diet and hypercholesterolemia on sperm function and quality has been studied and reported both in animal and human studies. In human, hypercholesterolemia independent of obesity was found to be negatively associated with sperm morphology and vitality (Schisterman *et al.*, 2014). In animals, hypercholesterolemia caused reduction in the sperm count, motility and vitality associated with an increase in the sperm morphological abnormalities (Saez Lancellotti *et al.*, 2010, 2013; Ouvrier *et al.*, 2011). However, for the first time, this study demonstrated that honey supplementation—using Trihoney—recovered almost all of the changes in sperm parameters induced by the high cholesterol diet.

The results of the effects of 1% cholesterol diet, Trihoney and atorvastatin on the different categories of sperm motility are summarized in Table 2.

Table 2. Effects of 1% cholesterol diet, Trihoney and atorvastatin on percentages of sperm motility

Parameters Groups	PM (%)	TM (%)	Immotile sperm (%)
C	85.38±7.96 ^a	96.40±1.16 ^a	3.60±1.16 ^a
CH	82.72±15.49 ^a	95.73±4.05 ^a	4.27±4.05 ^a
HCD	15.88±12.23 ^b	60.42±30.69 ^{b,c}	39.58±30.69 ^{b,c}
DAt	42.36±40.47 ^{b,c}	54.16±49.24 ^b	45.84±49.24 ^b
DH1	58.32±34.32 ^{a,c}	79.43±27.34 ^{a,c}	20.56±27.34 ^{a,c}
DH2	75.20±20.44 ^a	94.20±3.26 ^a	5.80±3.26 ^a

Data were analysed using one-way analysis of variance. A Post Hoc test (LSD) was used to test the significant difference between the groups. Values are given as mean ± standard deviation. ^{a,b,c} Values not sharing a common superscript letter within the same column differ significantly at p<0.05. PM: progressive motility; TM: total motility. C: commercial pellet; CH: commercial pellet plus 600 mg/kg/day Trihoney; HCD: 1% cholesterol diet; DH1: 1% cholesterol diet plus 300 mg/kg/day Trihoney; DH2: 1% cholesterol diet plus 600 mg/kg/day Trihoney; DAT: 1% cholesterol diet plus 2 mg/kg/day atorvastatin.

Motility is an important characteristic of fertile sperm. It is the most widely used indicator of sperm function as it reflects structural and functional competence of the sperm (Partyka *et al.*, 2012). The extent of progressive motility is related to pregnancy rate (WHO, 2010). Only sperm moving in straight patterns succeed in the process of fertilization; whereas, those swim in tight circles are unable to pass through the utero-tubal junction (Amelar *et al.*, 1980). In the present study, administration of a high cholesterol diet induced reduction in the percentages of progressive (p<0.05) and total motility (p<0.05) and increased the percentage of immotile sperm (p<0.05) compared to the control group. In agreement with this study, Saez Lancellotti *et al.* (2010, 2013) and Yamamoto *et al.* (1999) reported the same effects of a hypercholesterolemic diet on sperm motility in rabbits. The negative impact of a high cholesterol diet on sperm motility was also reported in transgenic mice (Ouvrier *et al.*, 2011).

Normal morphology is another important attribute of sperm and its evaluation is essential in sperm analysis. It provides clinical information about the potential fertility of semen sample (Partyka *et al.*, 2012). The alteration of normal sperm morphology could be a sign of subfertility (Saez Lancellotti *et al.*, 2010). Cholesterol intake promotes several changes in sperm cells (Saez Lancellotti *et al.*, 2013). In the

current study, a high cholesterol diet significantly decreased the percentage of sperm with normal morphology ($p < 0.05$) and increased the percentage of sperm with abnormal morphology ($p < 0.05$) as demonstrated in Table 3 and Figure 1. Similar results were reported by Saez Lancellotti *et al.* (2010, 2013). In those studies, the morphological abnormalities were in the form of folded head and presence of cytoplasmic droplets; however, the morphological alterations in the present study were mostly in the form of coiled or bent tail, bent neck and double tails. The morphological abnormalities which were reported in our study are in agreement in part with another study conducted by Shalaby (2004) to study the effect of α -tocopherol and simvastatin on infertility in hypercholesterolemic rats. Ouvrier *et al.* (2011) demonstrated an increase in the percentage of broken cells with high cholesterol diet.

Table 3 also demonstrates the effects of 1% cholesterol diet on sperm concentration. Sperm concentration is used to measure the ability of the testes to produce an adequate number of sperm and the patency of the male reproductive tract. It is related to fertilization and pregnancy rate and is a predictor of pregnancy (WHO, 2010). In this study, administration of 1% cholesterol diet caused severe

reductions in the sperm concentration ($p < 0.05$). This result is in line with the results reported by Saez Lancellotti *et al.* (2013) and Shalaby (2004). However, Ouvrier *et al.* (2011) reported that there is no difference in epididymal sperm count after a high cholesterol diet administration, which was attributed to the normal testicular structure and function in that study.

Another essential parameter of potentially fertile semen sample is the viable sperm (Martínez, 2004). The effects of 1% cholesterol diet on sperm vitality are given in Table 3 and Figure 1. Even though there was a reduction in the percentage of live sperm after a high cholesterol diet administration in the present study, it lacks the significant difference from the control. Similar results were obtained by Saez Lancellotti *et al.* (2013) upon administration of a high cholesterol diet to male rabbits. Contradicting results exhibiting a significant reduction in sperm vitality after a high cholesterol diet administration were reported in previous studies conducted on mice and rats, which was suggested to be due to the earlier effect of the high cholesterol diet on the epididymal tissue than its effect on the testicular tissue (Ouvrier *et al.*, 2011; Shalaby, 2004).

Table 3. Effects of 1% cholesterol diet, Trihoney and atorvastatin on percentages of sperm vitality, sperm morphology and sperm concentration

Parameters	Live sperm	Dead sperm	Normal sperm	Abnormal sperm	Concentration
Groups	(%)	(%)	(%)	(%)	(Sperm $\times 10^6$ /mL)
C	89.50 \pm 4.51 ^a	10.50 \pm 4.51 ^a	95.25 \pm 3.13 ^a	4.75 \pm 3.13 ^a	85.93 \pm 44.21 ^a
CH	89.67 \pm 3.53 ^a	10.33 \pm 3.53 ^a	92.08 \pm 8.07 ^a	7.92 \pm 8.07 ^a	86.18 \pm 33.29 ^a
HCD	64.80 \pm 32.03 ^{a,b}	35.20 \pm 32.03 ^{a,b}	64.22 \pm 25.23 ^b	35.78 \pm 25.23 ^b	12.43 \pm 12.74 ^b
DAt	55.00 \pm 44.85 ^b	45.00 \pm 44.85 ^b	63.60 \pm 39.89 ^b	36.40 \pm 39.38 ^b	18.94 \pm 24.23 ^b
DH1	76.75 \pm 30.59 ^{a,b}	23.25 \pm 30.59 ^{a,b}	82.33 \pm 16.06 ^{a,b}	17.67 \pm 16.06 ^{a,b}	37.71 \pm 43.59 ^b
DH2	86.58 \pm 6.75 ^a	13.42 \pm 6.75 ^a	91.00 \pm 4.87 ^a	9.00 \pm 4.87 ^a	45.46 \pm 18.90 ^b

Data were analysed using one-way analysis of variance (ANOVA). A Post Hoc test (LSD) was used to test the significant difference between the groups. Values are given as mean \pm standard deviation of mean. ^{a,b} Values not sharing a common superscript letter within the same column differ significantly at $p < 0.05$. C: commercial pellet; CH: commercial pellet plus 600 mg/kg/day Trihoney; HCD: 1% cholesterol diet; DH1: 1% cholesterol diet plus 300 mg/kg/day Trihoney; DH2: 1% cholesterol diet plus 600 mg/kg/day Trihoney; DAt: 1% cholesterol diet plus 2 mg/kg/day atorvastatin.

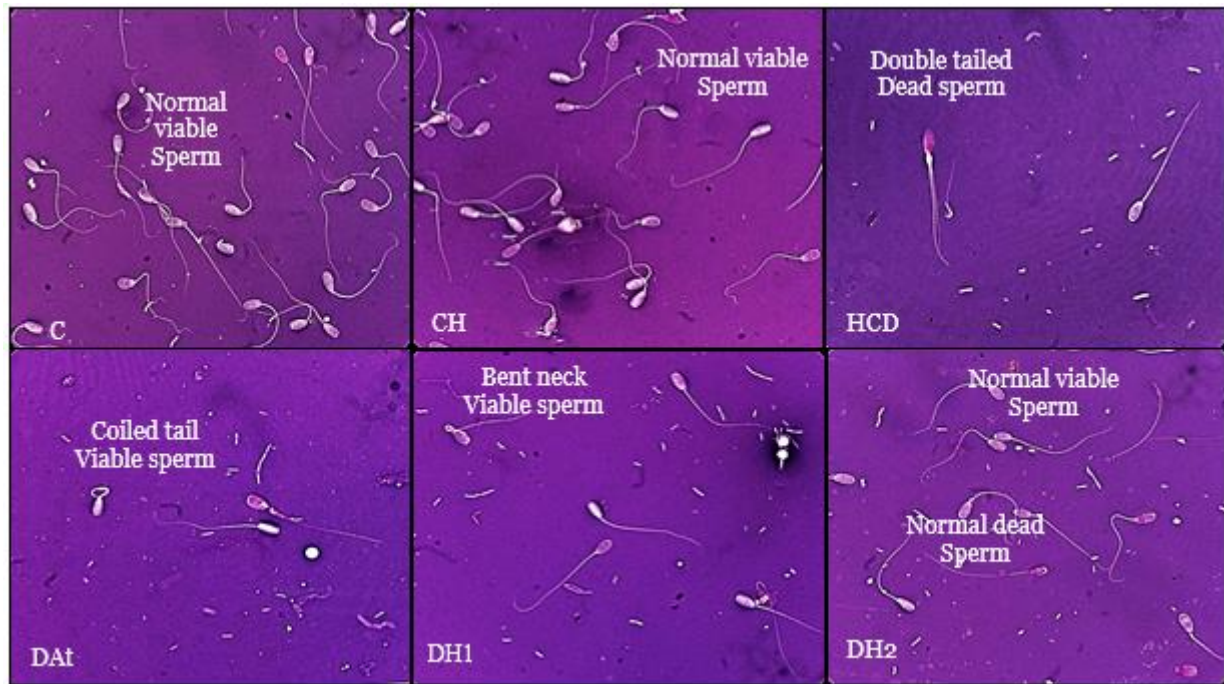


Figure 1. Eosin-Nigrosin stain of sperm morphology and vitality. The smears of the slides from the different groups were examined with $\times 1000$ magnification (oil lens). C: commercial pellet; CH: commercial pellet plus 600 mg/kg/day Trihoney; HCD: 1% cholesterol diet; DH1: 1% cholesterol diet plus 300 mg/kg/day Trihoney; DH2: 1% cholesterol diet plus 600 mg/kg/day Trihoney; DAt: 1% cholesterol diet plus 2 mg/kg/day atorvastatin.

There was no significant difference between CH group and the control group in any of the tested sperm parameters. These comparable results indicate that Trihoney has no negative impacts on sperm parameters. It also showed that Trihoney has no enhancing effects on these tested parameters in the normocholesterolemic rabbits.

The deleterious impacts of a high cholesterol diet and hypercholesterolemia on sperm quality parameters could be induced *via* their negative impacts on testicular tissue. Hypercholesterolemia induces testicular degenerative changes that render seminiferous tubules atrophied and non-functioning with arrest of spermatogenesis (Mohamed *et al.*, 2020a). These degenerative changes possibly caused by an oxidative stress damage (Ashrafi *et al.*, 2013; Shalaby, 2004). High cholesterol diet and hypercholesterolemia interrupt hypothalamic-pituitary-gonadal axis with a subsequent disruption of spermatogenesis (Hamad Mohamed *et al.*, 2020; Bashandy, 2007). The reduction in the sperm concentration and motility in a high cholesterol diet fed-animals may be attributed to the impairment of Sertoli and Leydig cells secretory function which subsequently affects spermatogenesis and epididymal sperm maturation. Epididymal dysfunction is another contributory factor in the

reduction of sperm motility and cytostructural modifications that affect sperm morphology (Yamamoto *et al.*, 1999). The epididymis was suggested as an early target of dietary cholesterol overload. The alteration induced by hypercholesterolemia in epididymal function causes a dramatic reduction of sperm motility and vitality and an increase in sperm morphological abnormalities (Ouvrier *et al.*, 2011).

Concurrent supplementation of Trihoney with a high cholesterol diet improved the percentages of sperm progressive motility ($p < 0.05$), total motility ($p < 0.05$) and sperm with normal morphology ($p < 0.05$) and reduced the percentages of immotile sperm ($p < 0.05$) and sperm with abnormal morphology ($p < 0.05$) when compared to the high cholesterol diet group; meanwhile, their levels were comparable to the control as illustrated in Tables 2 and 3 and Figure 1. More improvements were achieved by the Trihoney dose of 600 mg/kg/day than the dose of 300 mg/kg/day. Even though sperm concentration increased with Trihoney supplementation, it remained significantly lower than the control ($p < 0.05$). To the best of our knowledge, no previous study has been conducted to determine the effects of honey against the changes in sperm parameters that are induced by

a high cholesterol diet. However, there are numerous studies that have been conducted to determine the importance of honey in abating the impacts of other toxicants on sperm parameters. Administration of honey to rats exposed to cigarette smoke improved sperm count, percentage of motile sperm and the percentage of sperm with normal morphology (Mahaneem *et al.*, 2011). Supplementation of honey to nicotine-treated male rats improved fertility through improvements in sperm concentration, percentage of motile sperm and the number of normal sperm (Aisyah *et al.*, 2011). The same effects on sperm parameters were achieved by honey in rats fed with a high sucrose diet (Oyelowo *et al.*, 2014) and in diabetic rats (Michael *et al.*, 2015). Moreover, sperm progressive motility and post insemination pregnancy rate in mice were improved upon addition of 10% of honey to IVF media (Hadi, 2017). In human, honey supplementation to oligospermic men improved sperm concentration, motility and morphology (Ismail *et al.*, 2014). Supplementation of 10% of honey to cryoprotectant solution results in an enhancement of sperm quality post-thawing (Fakhrildin & Alsaadi, 2014).

The likely underlying mechanisms behind the positive effects of Trihoney on sperm parameters of high cholesterol diet-fed rabbits are its ability to maintain testicular integrity and optimize neuroendocrine gonadal axis and in turn enhancement of spermatogenesis, which were suggested to be due to Trihoney antioxidant activity as published recently (Hamad Mohamed *et al.* 2020; Mohamed *et al.*, 2020a). The antioxidant activity of honey reduces lipid peroxidation and restores the antioxidant system of the testicular tissue (Mohamed *et al.*, 2011).

The other possible mechanisms could be *via* enhancement of spermiogenesis (Mohamed *et al.*, 2012) and spermatogenesis through the decrease in testicular lactate dehydrogenase and increase in sorbitol dehydrogenase which are testicular marker enzymes for spermatogenesis (Abdul-Ghani *et al.*, 2008). It possible that honey acts as a physiologic modulator of spermatogenic cells proliferation which influences the cell cycle of the seminiferous epithelium and increases spermatogenesis (Syazana *et al.*, 2011). Furthermore, honey enhances spermatogenesis and sperm vitality *via* abating apoptosis and necrosis in testicular cells which could be due to the antioxidant properties of honey.

The antioxidant activity may neutralize the patterns of expression of apoptosis related genes and proteins or controlling anti-apoptotic patterns (Saki *et al.*, 2013). Honey is a source of glucose, fructose, minerals and vitamins that stimulates sperm motility. Glucose and fructose are considered to be a fuel source for sperm motility (Hadi, 2017).

Treatment of the animals with atorvastatin showed better percentages of sperm PM and sperm concentration than using a high cholesterol diet alone; however, it did not reach the statistical significance. For percentages of sperm total motility, immotile sperm, vitality and normal morphology, atorvastatin treatment caused the worst results among the groups including the group that received high cholesterol diet alone. In a study carried out on healthy men, atorvastatin treatment resulted in a reduction of sperm concentration and vitality coupled with an increase in the percentage of abnormal sperm. Conversely, total sperm motility showed a significant improvement with no effects of atorvastatin observed on progressive motility (Pons-Rejraji *et al.*, 2014). Use of atorvastatin in the treatment of hypercholesterolemic patients has been noticed as a cause of reduction of sperm motility. This was suggested to be due to the inhibition of coenzyme Q (10) (CoQ10) (Niederberger, 2005). Inhibition of cholesterol biosynthesis also inhibits the synthesis of CoQ10 as they share the same biosynthetic pathway. A brief exposure to atorvastatin causes a marked reduction of CoQ10 (Rundek *et al.*, 2004). The negative impact of atorvastatin on sperm parameters could also be through reduction of serum and/or testicular testosterone. The reduction of serum and testicular testosterone with atorvastatin was reported both in animal and human studies (Hamad Mohamed *et al.*, 2020; Bustan & Jawad, 2017; Kanat *et al.*, 2009). Atorvastatin was suggested as one of the statins that might induce primary hypogonadism even at low doses (Corona *et al.*, 2010). Statins reduce testosterone *via* reduction of cholesterol, inhibition of steps of steroidogenesis process or inhibition of *de novo* synthesis of cholesterol in Leydig cells (Schooling *et al.*, 2013).

IV. CONCLUSION

Trihoney showed its effectiveness as a potential protective natural product that minimizes the negative impacts of a high cholesterol diet on sperm quality parameters. The protective

effects of Trihoney might be attributed to its high antioxidants constituents, which is suggested to be explored in future studies. On the other hand, treatment of rabbits with atorvastatin failed to reverse any of the changes induced by the high cholesterol diet in the sperm parameters.

V. REFERENCES

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