ORIGINAL ARTICLE

Effect of Trihoney (A Mixture of Trigona, Mellifera and Tualang) on Male Reproductive Hormones and Insulin Resistance in Hypercholesterolaemic Rabbits

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ABSTRACT

Introduction: Hypercholesterolaemia affects Sertoli and Leydig cells secretory functions, impairs steroid hormones biosynthesis, and disrupts the hypothalamic-pituitary-gonadal axis. The use of honey in previous studies resulted in an improvement of male reproductive hormonal disturbances. This study aimed to investigate the protective effects of Trihoney on hypercholesterolaemia-induced male reproductive hormonal changes in male rabbits and compare its effects with atorvastatin. **Materials and methods:** Forty-eight male New Zealand white rabbits were assigned into 6 groups as follows; Control: commercial pellet; CH: commercial pellet with 0.6 g/kg/day Trihoney; HCD: 1% cholesterol diet; DH1: 1% cholesterol diet with 0.3 g/kg/day Trihoney; DH2: 1% cholesterol diet with 0.6 g/kg/day Trihoney and DAt: 1% cholesterol diet with 2mg/kg/day atorvastatin. After 12 weeks, the rabbits were sacrificed and blood samples were collected for analysis of hormones and pro-inflammatory cytokines and calculation of HOMA-IR. The testes were homogenized for intra-testicular testosterone measurement. **Results:** Serum testosterone reduced significantly in HCD ($p<0.05$) and DAt ($p<0.05$) groups. Likewise, intra-testicular testosterone reduced significantly in HCD ($p<0.01$) and DAt ($p<0.01$) groups. Serum FSH increased significantly in HCD ($p<0.001$) and DAt ($p<0.01$). Trihoney particularly at the dose of 0.6 g/kg/day improved serum and intra-testicular testosterone ($p<0.05$) and FSH ($p<0.05$). Trihoney and atorvastatin improved serum pro-inflammatory cytokines. Trihoney and atorvastatin did not affect HOMA-IR. **Conclusion**: Trihoney attenuated the detrimental effects of hypercholesterolaemia on male reproductive hormones which probably through a local effect on testicular tissue and Trihoney anti-inflammatory effect independent of insulin resistance. Atorvastatin did not counteract the impact of hypercholesterolaemia on the reproductive hormones.

KEYWORDS: Hypercholesterolaemia; Trihoney; Atorvastatin; Male reproductive hormones; Insulin resistance

INTRODUCTION

Testosterone is the principal male sex hormone secreted by Leydig cells in the testes. It is essential for testes development, spermatogenesis, and masculinization. Testosterone plasma level is a useful indicator of testicular function.[1] Inside Sertoli cells, testosterone is bound to androgen receptors. Activation of these receptors results in initiation and maintenance of spermatogenesis and prevents germ cells apoptosis.[2] Follicle-stimulating hormone (FSH) and luteinizing hormone (LH) are secreted from the anterior pituitary gland and they are important for the normal male reproductive system. Follicle-stimulating hormone stimulates spermatogenesis while LH triggers synthesis and secretion of androgens mainly testosterone.[3] Previous studies have demonstrated a strong influence of dietary

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factors on testosterone with high-energy diet is one of the factors which may cause an imbalance in serum and intra-testicular testosterone. This imbalance was suggested to be due to the low level of insulin that negatively affects testosterone secretion.[4] Systemic inflammation and overproduction of inflammatory cytokines are leading causes to the reduction of androgen production with subsequent disruption of spermatogenesis. Inconsistent results were reported about the effects of the high-energy diet and hypercholesterolaemia, whether it is associated with obesity or not, on the hormonal profile of the male reproductive system.

While some studies revealed an increase in testosterone, FSH, and LH,[5] others demonstrated a reduction in the levels of these hormones.[4,6,7] On the other hand, the use of honey in previous studies resulted in an improvement of the male reproductive hormonal disturbances through increasing testosterone and reducing FSH and LH in rats fed with high sucrose diet and rats exposed to noise stress.[8,9] Trihoney is a product developed by the Department of Nutrition Sciences of Kulliyyah of Allied Health Sciences of International Islamic University Malaysia. It is a mixture of Trigona, Mellifera and Tualang honey at a ratio optimized by Response Surface Methodology of Design Expert Version 6.0 software. This ratio produces the highest result of total phenolic content with consuming the minimum amount of each honey. Thus, the purpose of the current study was to investigate the protective effect of Trihoney on male reproductive hormonal changes induced by hypercholesterolaemia in male rabbits and compare its effect with atorvastatin as the most commonly used hypolipidaemic agent.

MATERIALS AND METHODS

Animal

Forty-eight male New Zealand white rabbits of 5-months age were used in this study. Their environment was maintained under controlled conditions of 15-21°C temperature, 45-65% humidity, and 12hours light/dark cycles. The rabbits were caged individually and provided free access to water and food. The animal handling procedures, treatment, and experimental protocols were approved by the International Islamic University Malaysia Institutional Animal Care and Use Committee (IACUC-IIUM), IIUM, Kuantan campus [No. of IACUC Approval: IIUM/IACUC Approval/ 2017/ (18) -2].

Preparation of 1% cholesterol diet

One percent cholesterol diet was prepared according to the method of Mohamed et al., (2020).[10] Ten grams of cholesterol powder (Nacalai-Tesque, Japan) were emulsified in 20mL (2%) of coconut oil (Certified Organic, Philippines). The emulsion was then poured onto 970 grams of rabbit pellet and mixed thoroughly. It was repeatedly and freshly prepared twice weekly.

Trihoney and atorvastatin doses

Trihoney doses were calculated based on the conversion of human equivalent dose to rabbit equivalent dose according to the following equation:[11]

Human equivalent dose × Human K*m* factor= Animal equivalent dose × Animal K*m* factor

The used dose of atorvastatin (Atorvastatin Winthrop®, Prague-Czech) was 2mg/kg/day.[12] Atorvastatin was reconstituted in 1mL of distilled water then administered orally.[13]

Experimental design

After 2 weeks of acclimatization, the rabbits were assigned into 6 groups (n=8) and received different diets as follows: 1) Control (C): commercial rabbit pellet, 2) CH: commercial pellet with a daily oral dose of 0.6 g/kg/day Trihoney, 3) HCD: 1% cholesterol diet, 4) DH1: 1% cholesterol diet with a daily oral dose of 0.3 g/kg/day Trihoney, 5) DH2: 1% cholesterol diet with a daily oral dose of 0.6 g/kg/ day Trihoney, 6) DAt: 1% cholesterol diet with a daily oral dose of 2mg/kg/day atorvastatin. Their weights were measured once weekly and the doses were adjusted accordingly.[5] The duration of the experiment was 12 weeks.

Animal sacrificing, blood collection and serum separation

At the end of the 12 weeks, the rabbits fasted overnight and were euthanized using intramuscular injection of ketamine/ xylazine at a dose of 50/10 mg/kg body weight followed by exsanguination.[14,15]

Blood samples were collected from the central ear artery for analysis of hormones (FSH, LH, and testosterone), fasting insulin, fasting blood glucose, and pro-inflammatory cytokines. The blood was allowed to clot at room temperature before centrifugation (Centrifuge Universal 320R, Hettich) at 3500g and 4°C for 15 minutes to separate the serum which was stored at −80°C till the time of analysis.[16–18] Fasting blood glucose and fasting insulin were measured using an automated analysis machine (AU 480 AU Analyser-Beckman Coulter, Inc. USA). Homeostatic model assessment of insulin resistance (HOMA-IR) was calculated using Matthews et al. (1985)[19] equation as follows:

HOMA-IR= Fasting serum insulin (μU/mL) × Fasting plasma glucose (mmol/L)/22.5

Testes harvesting

Under clean conditions, a 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.[20] Systemic perfusion was performed using ice-cold normal saline at a flow rate of 200 mL/minute through the left ventricular approach. The left testis was then excised, cut into pieces and preserved at −80°C for homogenate preparation.[21]

Preparation of testicular tissue homogenate

Testicular tissue of each animal was weighed and homogenized to make 10% homogenate (w/v)[22] in ice-cold phosphate buffer saline (PBS)[23] using Bullet Blender blue homogenizer (Next Advance, USA). Twenty microliters of protease inhibitor cocktail (Nacalai Tesque, Japan) was added to each testicular homogenate. The homogenate was then centrifuged in a refrigerated centrifuge (ThermoFisher Scientific, Germany) at 10000g for 15 minutes at 4°C. The obtained supernatant was stored at −80°C and used for assaying of protein concentration and intra-testicular testosterone.

Protein assay in the testicular homogenate

Protein concentration in the testicular homogenate was calculated to be used as a reference unit for the concentration expression of the assayed intra-testicular testosterone. It 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.[22]

Analysis of hormones and pro-inflammatory cytokines

Serum and intra-testicular testosterone, serum FSH and LH and serum interleukin (IL)-1β were estimated using Elisa kits from Cusabio Biotech, China. Serum IL-6 and tumour necrosis factor-alpha (TNF-α) were estimated using Elisa kits from Elabscience, China. The analysis was done according to the manufacturer's instructions.

Statistical analysis

Data were processed using the Statistical Package for the Social Sciences Version 21 (SPSS Inc., Chicago, Illinois, USA) program. 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.

RESULTS

Effects of hypercholesterolaemia, Trihoney, and atorvastatin on serum and intra-testicular testosterone

Table I demonstrates the effects of hyper-cholesterolaemia, Trihoney, and atorvastatin on serum and intra-testicular testosterone. There was no significant difference between the CH group and the control in the serum and intra-testicular testosterone. Administration of 1% cholesterol diet either alone or in combination with atorvastatin showed a significant reduction in the serum ($p<0.05$) and intra-testicular ($p<0.01$) testosterone when compared to the control group. Trihoney received groups showed no significant difference from the control group in serum testosterone. The DH1 group was significantly lower than the control in intra-testicular testosterone ($p<0.01$). There was no significant difference between DH2 and the control group in intra-testicular testosterone. The DH2 exhibited higher levels of serum ($p<0.05$) and intra-testicular ($p<0.05$) testosterone than the HCD group.

Serum and intra-testicular testosterone were higher in DH1 than HCD and DAt groups but no significant difference was detected among them. The DH2 group expressed a significantly higher level of intra-testicular testosterone than the DAt group ($p<0.05$). There was no significant difference between DH1 and DH2.

Table I: Effects of hypercholesterolaemia, Trihoney and atorvastatin on serum and intra-testicular testosterone levels

Hormones Groups	Serum testosterone (ng/mL)	Intra-testicular testosterone (ng/mg protein)
C	6.23±0.87	0.34±0.02
CH	6.66±0.85	0.35±0.01
HCD	2.28±1.90[a*]	0.26±0.05[a**]
DAt	2.92±1.54[a*]	0.26±0.01 [a**]
DH1	5.43±1.76	0.28±0.04 [a**]
DH2	6.05±4.19 [b*]	0.31±0.04 [b*,c*]

Data were analysed using a one-way analysis of variance (ANOVA). A Post Hoc comparison using the LSD test was used to test the significant difference between the groups. Values are given as mean ± standard deviation of the mean. [a]:significantly different from control; [b]:significantly different from the HCD group. [c]: significantly different from DAt group *:p<0.05; **:p<0.01. 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.

Effects of Hypercholesterolaemia, Trihoney, and Atorvastatin on Serum Follicle-stimulating Hormone and Luteinizing Hormone

Effects of hypercholesterolaemia, Trihoney, and atorvastatin on serum FSH and LH are given in Table II. There was no significant difference between the CH group and the control group in the serum levels of FSH and LH. A marked elevation in serum FSH was expressed by the HCD group compared to the control group ($p<0.001$). Atorvastatin received group exhibited a significantly higher level of FSH than the control group ($p<0.01$). There was no significant difference between the HCD group and the DAt group. The DH1 ($p<0.01$) and DH2 ($p<0.05$) demonstrated significantly higher levels of FSH compared to the control group. A significantly lower level of FSH was shown by DH2 compared to the HCD group ($p<0.05$). There was no significant difference between DAt, DH1, and DH2. As per LH, no significant difference was detected neither between the treatment groups and the control group nor among the treatment groups.

Table II: Effects of Hypercholesterolaemia, Trihoney, and Atorvastatin on Serum Follicle-stimulating Hormone and Luteinizing Hormone

Hormones Groups	FSH (mIU/mL)	LH (mIU/mL)
C	5.54±0.28	2.59±0.51
CH	5.37±1.06	2.52±0.77
HCD	12.27±2.75[a***]	3.54±0.44
DAt	9.72±1.95 [a**]	2.89±0.72
DH1	9.88±0.89 [a**]	3.02±1.29
DH2	8.81±2.52 [a*, b*]	2.98±0.69

Data were analysed using a one-way analysis of variance (ANOVA). A Post Hoc comparison using the LSD test was used to test the significant difference between the groups. Values are given as mean ± standard deviation. [a]:significantly different from control; [b]:significantly different from the HCD group. *:p<0.05; **p<0.01; ***:p<0.001. 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; FSH: follicle-stimulating hormone; LH: luteinizing hormone.

Effects of hypercholesterolaemia, Trihoney, and atorvastatin on fasting blood glucose, fasting serum insulin and HOMA-IR

Table III illustrates the results of fasting serum insulin, fasting blood glucose, and HOMA-IR. There was no significant difference neither between the treatment groups and the control group nor among the treatment groups in these parameters.

Table III: Effects of hypercholesterolaemia, Trihoney, and atorvastatin on fasting blood glucose, fasting serum insulin and HOMA-IR

Parameters Groups	Fasting glucose (mmol/L)	Fasting insulin (mU/L)	HOMA-IR
C	4.80±0.92	0.41±0.28	0.09±0.06
CH	4.90±1.16	0.48±0.17	0.10±0.04
HCD	5.30±1.95	0.51±0.29	0.14±0.14
DAt	6.03±1.12	0.47±0.08	0.12±0.02
DH1	5.75±2.21	0.56±0.15	0.15±0.06
DH2	6.79±1.25	0.50±0.14	0.15±0.06

Data were analysed using a one-way analysis of variance (ANOVA). A Post Hoc comparison using Tukey HSD test was used to test the significant difference between the groups. Values are given as mean ± standard deviation of the mean. 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 2mg/kg/day atorvastatin.

Effects of 1% cholesterol diet, Trihoney, and atorvastatin on serum pro-inflammatory cytokines

The effects of 1% cholesterol diet, Trihoney, and atorvastatin on the serum pro-inflammatory cytokines are given in Table IV. There was no significant difference in the serum pro-inflammatory cytokines between the CH group and the control group. The HCD group showed significantly higher levels of TNF-α ($p<0.05$) and IL-1β ($p<0.001$) than the control group with a non-significant increase in IL-6. Treatment with atorvastatin caused a significant reduction in the serum TNF-α ($p<0.01$) and IL-1β ($p<0.01$) when compared to the HCD group with a non-significant reduction in IL-6.

Trihoney received groups showed significantly lower levels of serum TNF-α ($p<0.001$) than the HCD group and control group ($p<0.05$ for DH1 and $p<0.01$ for DH2). The DH1 ($p<0.01$) and DH2 ($p<0.05$) were significantly lower than the HCD group in the serum IL-1β and IL-6 ($p<0.05$). There was no significant difference among DAt, DH1, DH2, and the control group in the serum IL-1β and IL-6. No significant difference was found between Trihoney received groups and atorvastatin treated group in the serum TNF-α level.

Table IV: The effect of 1% cholesterol diet, Trihoney and atorvastatin on the serum pro-inflammatory cytokines

Parameters Groups	TNF-α (pg/mL)	IL-6 (pg/mL)	IL-1β (pg/mL)
C	15.09± 0.32	6.37± 0.68	85.99± 5.84
CH	14.22± 0.24	6.57± 0.34	83.08± 10.42
HCD	16.64± 2.09[a*]	7.88± 1.79	167.07± 13.36[a***]
DAt	13.91± 0.18 [b**]	6.96± 2.00	96.15± 23.90 [b**]
DH1	13.51± 0.08 [a*,b***]	5.96± 0.47[b*]	115.77± 43.85 [b**]
DH2	12.74± 1.37 [a**,b***]	5.91± 0.41 [b*]	130.31± 28.05 [b*]

Data were analysed using a one-way analysis of variance (ANOVA). A Post Hoc comparison using the LSD test was used to test the significant difference between the groups. Values are given as mean ± standard deviation of the mean. [a]:significantly different from control; [b]: significantly different from the HCD group. *: $p<0.05$; **: $p<0.01$; ***:$p<0.001$. 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; IL: interleukin; TNF-α: tumour necrosis factor-alpha.

DISCUSSION

In this study, Trihoney particularly at the dose of 0.6 g/kg/day improved serum and intra-testicular testosterone, serum FSH, and serum pro-inflammatory cytokines. Treatment with atorvastatin improved serum pro-inflammatory cytokines but did not counteract the impact of hypercholesterolaemia on the reproductive hormones. Feeding of rabbits with a 1% cholesterol diet either alone or in combination with Trihoney or atorvastatin did not affect the levels of fasting serum insulin, fasting blood glucose, and HOMA-IR.

Gonadotropin-releasing hormone, FSH, LH, and testosterone are the hormones of the hypothalamic-pituitary-testicular axis. They are the main regulatory hormones of sperm production.[24] Disturbance of this tightly regulated axis disrupts the normal physiology, metabolism and bioenergetics capacity of testicular tissue with a subsequent impairment of male fertility.[25]

In this study, the reduction in serum testosterone coupled with an elevation of FSH indicates that a testicular dysfunction (primary testicular failure) was induced by hypercholesterolaemia.[26] Hypercholesterolaemia affects testicular structure and function, Sertoli and Leydig cells secretory functions, impairs steroid hormones biosynthesis and disrupts hypothalamic-pituitary-gonadal axis.[27] Numerous studies have been conducted to investigate the effects of the high-energy diet and hypercholesterolaemia/hyperlipidaemia on male reproductive hormones. Zarei et al. (2014)[7] reported that feeding of rats with a 2% cholesterol diet for 3 weeks significantly reduced serum FSH with no significant change in the serum testosterone and LH. Alzubaidi & Diwan (2013)[30] revealed that feeding of rats with a 1.5% cholesterol diet for 4 weeks caused a severe reduction in serum testosterone, FSH, and LH. Induction of hypercholesterolaemia, accompanied by hypertriglyceridaemia and obesity, in rabbits in a previous study produced a significant increase in plasma testosterone and FSH with a slight increase in LH and a reduction in intra-testicular testosterone.[5] Rato et al. (2013)[4] demonstrated that supplementation of a high-energy diet containing cholesterol to rats resulted in a reduction of serum and testicular testosterone. The hormonal disturbances induced by hypercholesterolaemia in the

current study are likely caused by its effect on the testicular tissue rather than through an effect on the hypothalamus or pituitary gland. The increase in gonadotropin (FSH and to a less extent LH) is logically explained by the reduction in the serum testosterone which disrupts the feedback inhibitory mechanism on these hormones.[7] The increase in FSH can also be explained by impaired production of inhibin B from Sertoli cells due to testicular dysfunction.[28] Reduced serum testosterone and/or elevated serum FSH is indicative of impaired spermatogenesis and compromised fertility.[28,29] Additionally, the reduction in intra-testicular testosterone in the present study would have a detrimental effect on spermatogenesis as intra-testicular testosterone is vital for the maintenance of spermatogenesis.[2] The other possible mechanism of current hormonal disturbance is the elevation of serum pro-inflammatory cytokines by the administration of high-cholesterol diet. Overproduction of IL-1, IL-6, and TNF-α harms the male fertility through inhibition of steroidogenesis.[31-33] Systemic inflammation can cause male infertility through the reduction of androgen production with subsequent disruption of spermatogenesis.[34] Acute elevation of circulating IL-6 in healthy male volunteers through the injection of recombinant human IL-6 (rHuIL-6) induced prolonged reduction in testosterone secretion. As no effect was observed on the LH level, it was speculated that IL-6 might induce testicular resistance to LH effect or suppress testosterone production by Leydig cells.[33] A randomized controlled study showed that exposure to even minimal inflammatory stress through injection of interleukins reduced serum testosterone and amplified testosterone's negative feedback inhibition of LH.[35] To further support this relationship, IL-1β was found to be a potent down regulator of LH through a direct effect on the anterior pituitary gland during an inflammatory challenge in an *ex vivo* study.[36] In the current study, as there was a reduction in the testosterone with no significant changes in LH, it could be that the inflammatory cytokines induced a testicular resistance to LH or suppressed testosterone production by Leydig cells as suggested previously.[33]

Treatment of rabbits with atorvastatin improved serum pro-inflammatory cytokines but did not counteract the impact of hypercholesterolaemia on the reproductive hormones. The reduction of serum testosterone with atorvastatin was reported in animal and human studies.[37,38] Atorvastatin was one among other statins that were reported to reduce libido in men. Serum testosterone analysis of some of those reported cases revealed a significant reduction that regained to normal after statin withdrawal.[39] Atorvastatin was suggested as one of the statins that might induce primary hypogonadism even at low dose.[40] In a meta-analysis of randomized controlled trials, statins reduced testosterone level which was suggested to be through the reduction of cholesterol, the substrate of testosterone biosynthesis, or through inhibition of steps of steroidogenesis process.[41] It could also be through inhibition of *de novo* synthesis of cholesterol in Leydig cells.[39] This may explain the inability of atorvastatin to improve testosterone and subsequently FSH in the current study.

Supplementation of Trihoney particularly at the dose of 0.6 g/kg/day improved serum pro-inflammatory cytokines, serum and intra-testicular testosterone, and serum FSH. This hormonal improvement is probably due to the effect of Trihoney on testicular tissue. The improvement of testicular tissue increases testosterone production which, in turn, decreases FSH through the negative feedback mechanism. Improvement of testicular tissue can also increase inhibin B secretion which reduces FSH. The hormonal improvement could also be through the anti-inflammatory mechanism of Trihoney that reduced serum inflammatory biomarkers with a subsequent elevation in serum testosterone and reduction of FSH through the negative feedback mechanism of testosterone. The effect of honey on male reproductive hormones has been investigated in various studies but this is the first study attempts to determine the effect of honey on male reproductive hormonal disturbances induced by hypercholesterolaemia. Oyelowo et al. (2014)[9] reported that consumption of honey increased testosterone and reduced FSH and LH in rats fed with normal diet and rats received high sucrose solution. The beneficial effect of honey in increasing low serum testosterone, and reducing elevated serum FSH and LH was detected in noise-exposed rats.[8] Supplementation of honey to cigarette smoke-exposed rats, normal and diabetic rats improved serum testosterone without significant changes in serum FSH and LH. The improvement of serum testosterone was attributed to the local effect of honey on testicular tissue.[42,43] Supplementation of honey for 12 weeks did not affect serum testosterone, FSH and LH in oligospermia men.[44]

As per serum insulin and HOMA-IR, there are controversies about the significance and strength of the association between insulin and testosterone. Among men with low testosterone, some were found to have low while others have high insulin concentrations.[45] Insulin is deemed to have a regulatory role on serum testosterone concentration in men. Acute hyperinsulinaemia increased serum testosterone levels whereas, insulin suppression reduced testosterone in normal weight and obese individuals.[46,47]

Insulin is abundant in the central nervous system. The absence of its signaling in the hypothalamus disrupts the process of steroidogenesis through inhibition of Leydig cell development.[48] It has been suggested that insulin resistance could be a common underlying mechanism for both hypogonadisms and type 2 diabetes.[49] The reduction in testosterone concentration in this study cannot be explained by a disturbance in insulin concentration and sensitivity as the concentration and HOMA-IR were comparable among all of the groups.

CONCLUSION

Trihoney particularly at the dose of 0.6 g/kg/day significantly attenuated the detrimental effects of hypercholesterolaemia on male reproductive hormones. This indicates the potential health benefit of Trihoney to be used as a prophylactic agent to protect against hypercholesterolaemia-induced disruption of the male reproductive axis. The beneficial effect of Trihoney is most probably through a local effect on testicular tissue rather than on central regulatory mechanisms of male reproductive hormones. The second possible mechanism is the anti-inflammatory activity of Trihoney that reduced serum pro-inflammatory cytokines with a subsequent elevation in serum testosterone and reduction of FSH. Atorvastatin treatment did not show an improvement in the reproductive hormones.

Finally, the effects of hypercholesterolaemia, Trihoney, and atorvastatin on the male reproductive axis did not mediate through insulin concentration or insulin resistance as their results were compared among the groups.

CONFLICT OF INTEREST

The authors declared no conflict of interest

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