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GENDER DIFFERENCES ON THE EFFECTS OF HONEY AND BLACK SEED MIXTURE SUPPLEMENTATION

HAYATI MOHD YUSOF*, MOHAMAD AZLAN MANAN, NORIZAH MHD SARBON AND ASMA' ALI

School of Food Science and Technology, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia.

**Corresponding author: hayatimy@umt.edu.my*

Abstract: The effects of black seed and honey on cardiovascular disease (CVD) risk factors have been widely studied. However, few studies were done on its mixture especially on gender differences. Hence, this prospective open labelled supplementation study was aimed to investigate the effect of black seed and honey mixture (1:1 ratio) on CVD risk factors including lipid profile, blood pressure, blood glucose and hemoglobin, based on gender differences. Forty-one subjects recruited among Universiti Malaysia Terengganu staffs participated in this study, males (n=17) and females (n=23). Both groups were supplemented with black seed and honey mixture (2.3 g/day) for forty-five days (1.5 month). Venous bloods were taken before and after the study for analysis of lipid profile, hemoglobin and glucose concentrations. The supplementation shows a significant effect among gender. Females exhibit favorable effects on hemoglobin (p=0.007), systolic blood pressure (p=0.002), blood glucose (p=0.003) and total cholesterol (p=0.039). On the other hand, males show favorable effects on blood glucose (p=0.008) and total cholesterol (p=0.030). However, in terms of lipid profile, males tend to get more benefit following supplementation as compared to their female counterparts. Future investigations with larger sample size, longer duration period with several time points, dosage differences and effect of supplementation towards liver function and inflammatory markers are suggested to verify the present findings.

Keywords: Blood glucose, blood pressure, gender, honey and black seed mixture, lipid profile

Introduction

Cardiovascular disease (CVD) refers to arterial disease that restricting blood flow to the heart (coronary heart disease; CHD), or to the brain (cerebrovascular disease), or to the peripheral regions of the body (peripheral vascular disease; PVD) (Stanners, 2005). According to the WHO fact sheet (2015), CVD has accounted for nearly 17.5 million or 31% of total global deaths. Predominant of CVD includes people who are suffering from hypertension, heart disease, stroke, coronary heart disease (CHD), cerebrovascular disease and peripheral vascular disease, which is the leading cause of mortality (Siqueira *et al*., 2007; Roger *et al*., 2011). Until now, CVD still remains a leading cause of death in both developed and developing countries (Chia, 2011) and by 2020, it is predicted to be major causes of morbidity and mortality in most developing countries (Celermajer *et al*., 2012).

The current management to modify the CVD risk factors is through medication by drugs usage. To date, many drugs (especially statins) have been introduced and are available for the prevention and treatment of nearly all CVDs. However, a major problem with this drug usage is prolonged use has been claimed for side effects (Pahan, 2006). Therefore, an alternative treatment using natural remedies with minimum or no adverse effects are increasingly recognized. Thus, this study will focus on the beneficial effects of natural remedies using black seed and honey (in the form of mixture) in modulating the CVD risk factors.

Black seeds, also known as *Nigella sativa* is one of the herbs that was used a natural remedy to cure many diseases. The volatile oils in black seeds was shown to possess therapeutic potential on human health due to the presence of thymoquinone as a major bioactive compound (Ilaiyaraja & Khanum, 2010; Lutterodt *et al.,* 2010), dithymoquinone or 'nigellone', thymohdyroquinone and thymol (Ghosheh *et al.,* 1999). Borgou *et al.,* (2010) reported the potential of some terpenes (thymoquinones) as direct antioxidant, anti-inflammatory (p-cymene, cavracrol, ipinene, -terpinene and longifolone). Honey, on the other hand contains mainly fructose (38.5%), glucose (31.0%) and other sugars such as maltose and sucrose (12.9%) (Khan *et al.,* 2007). Several studies have found that the antioxidant activities of honey is related to the presence of phenolics (Khalil *et al.,* 2010; Koshore *et al*., 2011) especially phenolic acids, flavanoids, carotenoids (Bobis *et al.,* 2010) and other antioxidants including glucose oxidase, catalase, ascorbic acid and carotenoid derivatives (Khalil *et al.,* 2010). Thus, the anti-oxidative and anti-inflammatory compounds could be responsible for the hypolipidemic, hypotensive and hypoglcemic effects.

To date, studies have reported the hypolipidemic effects among hyperlipidemic patients (Tasawar *et al*., 2011; Mahmood *et al*., 2012), hypoglycemic effect among stable coronary artery patients (Sabzghabaee *et al*., 2012.), hypotensive effects among mild hypertensive patients (Dehkordi & Kamkhah, 2008) and hematologic effect among type 2 diabetic patients (Bamosa *et al*., 2010; Mohtashami *et al*., 2011) of black seed. Similarly, it has been reported that honey also shown beneficial effects on CVD risk factors including hypolipidemic effect among high cholesterol level respondents (Kei, 2014) and hypoglycemic effect among healthy individuals (Ahmad *et al*., 2008). However, for hypotensive and hematologic effects of honey, most studies were carried out in animals (Erejuwa *et al*., 2012; Aliyu *et al*., 2012). Honey also has been demonstrated to improve hemoglobin concentration among long history of AIDS patients (Al-Waili *et al*., 2006).

On the other hand, only limited studies on honey and black seed mixture reported its beneficial effects in improving the lipid profile and blood pressure among healthy and high blood cholesterol subjects (Yusof *et al*., 2012; Mohamad *et al*., 2014). Previously, duration of study on the effect of the honey and black seed supplementations was between two to three months. The dose used was between 2.8 - 3.6 g/day, thus, effect of supplementation at lower dosage is also un-documented and supplementation at duration of less than two months is unknown. Furthermore, limited studies investigated the effect of supplementation based on gender differences.

The general objective of the study was to investigate the effect of black seed and honey mixture supplementation at 2.3 g/day for 45 days (lower dosage and shorter duration) on CVD risk factors.

Materials and Methods

Black Seeds and Honey Mixture

In the present study, black seed was purchased from local market in Kuala Terengganu. Honey was purchased directly from local collector honey bees at Batu Rakit, Terengganu. The characteristics of honey are shown in Table 1. The mixture at 1:1 (about 1.15g *N. sativa* seeds and 1.15 g of honey) ratio of black seed and honey was prepared according to the procedure used by Yusof *et al*. (2012). Since the amount given was only 2.3 g/ day, the calorie content is negligible.

Table 1: Honey composition

Component	Results
Reducing sugar, g/100g:	
Fructose	40.46
Glucose	30.46
Maltose	0.47
Sucrose, g/100g	0.47
Ash, g/100g	0.17
Moisture, g/100g	25.80
Free acidity, meg/kg	67.30
Hidroxymethyl furfural, mg/kg	0.52
Diastase activity, DN	4.51

Subjects' Selection

In the present study, 91 adult's subjects were screened for eligibility criteria. Prior to the recruitment, they were provided with the subject information sheet. The subjects were selected with the following inclusion criteria: 25 – 58 years, free from chronic diseases such as hypothyroidism, diabetes mellitus, gastrointestinal disorder, renal impairment or cardiac problem such as heart attack or stroke, not treated with drugs (lipid-lowering drugs, anti-diabetic, aspirin) and did not consume any other dietary supplements, non-vegetarian, non-vigorous exercise (not more than 3 x 30 minutes vigorous sessions per week), non-pregnant or lactating, non-heavy smoker (not more than 10 cigarettes per day), non-blood donor in the last three months, not planned to lose weight and not participated in a clinical trial for last three months. Based on the sample size calculation, using the standard deviation 1.06 and the error of 0.53, with 95% confidence level the minimum sample size for each group is 15. This study was approved by the Universiti Sultan Zainal Abidin (UNiSZA) Human Research Ethics Committee (UHREC-628-1(42)) following the ethical consideration code for human.

Study Design

The subjects' eligibility was screened through Health and Lifestyle Questionnaire and their baseline data was taken in the first visit. Forty-one subjects were recruited and then divided into two groups: males (n=17) and females (n=23). A written informed consent form was obtained and all subjects were voluntarily decided to participate in this study. The measurements including blood pressure (BP), lipid profile (TC, TG, HDL-c, LDL-c), blood glucose and hemoglobin concentrations. The baseline measurements were taken in the first visit. The blood sample was collected in the morning, after the subjects had an overnight fasting for at least 10 hours and analyzed using validated and standardized instruments. Serum was obtained by blood centrifugation at 4000 rpm for 5 minutes. During the first visit, subjects were given the paste of black seed and honey mixture accordingly. They were required to consume the supplement daily according to the dosage instructed for forty-five days. During the supplemental period, subjects' cooperation were needed to maintain their usual daily lifestyles and also avoid the consumption of any other dietary supplements. The endline data was then collected in the second visit and the summary of study design is shown in Figure 1.

Blood Pressure

Systolic and diastolic blood pressure was measured from the left side arm using the Omron Automatic Blood Pressure Monitor, HEM-7080 model. The blood pressure was taken twice and an additional reading was done values from two consecutive measurements were more than 10 mm Hg apart. The measurement of systolic and diastolic blood pressure was taken after the subject sat for five minutes on the same arm (non-dominant) supported to heart level. The subject remained quiet (not talking, not laughing) and relaxed during the measurement process. The same Omron Automatic Blood Pressure Monitor was used throughout the study.

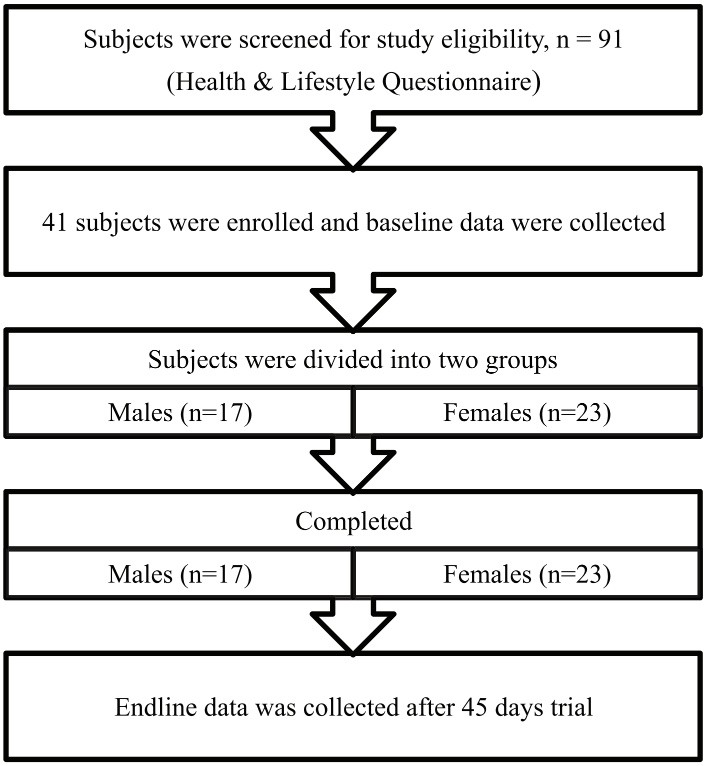

Figure 1: Study experimental design

Lipid Profile

Lipid profile measurements including total cholesterol, HDL-c and triglyceride were determined by using the Spotchem EZ SP-4430 Analyzer. Since LDL-cholesterol could not be measured directly, it was calculated based on Friedewald formula (Friedewald *et al*., 1972).

Blood Glucose and Hemoglobin

Fasting blood glucose level was determined directly using glucose reagent test strip with Accu-Check Advantage meter (Roche Diagnostics, Germany). Hemoglobin concentration was determined using the HemoCue® B-Hemoglobin microcuvettes with the HemoCue B-Hemoglobin Photometer (HemoCue AB, Sweden).

Statistical Analysis

All data were analyzed using statistical software SPSS version-22 (SPSS Inc., Chicago, IL). Shapiro Wilk test was applied for normality as the sample size in the present study was smaller than 100 (Garson, 2012). Normally distributed data in the present study for each group were expressed in mean ± SD values. Paired samples t-test and independent t-test were applied to determine differences in the measurement of baseline and after supplementation within the group and between groups, respectively. Wilcoxon Signed Rank test and Mann-Whitney test were used for non-parametric tests (Pallant, 2011). Data was presented as median (IQR) for not normally distributed data. A two-tailed, based on P values less than 0.05 were considered statistically significant for all the cases in the data analysis.

Results and Discussion

Baseline Characteristics of Subjects

In the beginning, a total of 41 subjects were recruited and agreed to participate in the study. Subjects were assigned into two groups; males (n=17) and females (n=23). Both groups were provided with 2.3 g of black seed and honey mixture for forty-five days (1.5 months). However, in the final analysis, one female subject was excluded due to unable to obtain her serum. The confirmed dosage was based on mean weight of the leftover supplement provided.

Table 2 shows the baseline characteristic of the subject. It was found that all parameters were not statistically different at baseline, apart from hemoglobin and HDL-c. The result shows that men had higher values for height (165.3 cm vs 153.5 cm; $p < 0.0001$) and hemoglobin (15.5 g/dL vs 12.9 g/ dL; $p < 0.0001$) than female respondents. On the other hand, females had a higher body fat percentage (34% vs 23%; $p < 0.0001$) and HDL-c (1.6 mmol/L vs 1.4 mmol/L; $p = 0.022$) as compared to males.

Effect of Black Seed and Honey Mixture on Blood Pressure

Table 3 shows the effect of supplementation towards systolic blood pressure (SBP) and diastolic blood pressure (DBP). Figure 2 shows comparison between groups at the end of intervention study in terms of changes (absolute change and percent change) in systolic blood pressure and diastolic blood pressure.

The result shows that only systolic blood pressure was decreased in female respondents from 111.50 mmHg to 110.50 mmHg. Lau (2010) found no significant effect of black seed and honey mixture supplementation (2.8 g/day for two months supplementation) on blood pressure in healthy subjects with study duration of two months. However, Mohamad *et al*. (2014) reported a significant blood pressure lowering effect using 3.8 g/day in a 3 month supplementation study in both systolic and diastolic blood pressures among high level cholesterol subjects. The present study (1.5 month) only shows favorable effect on systolic blood pressure among female respondent. Even though, statistically significant, in terms of clinical, this change is not apparent. Result

Table 2: Baseline characteristic of the subjects

Parameter	**Group**		**p-value**
	Male (n-17)	Female (n=23)	
Age (years)	39.00±9.86	38.00±8.15	0.709
Height (cm)	165.35±6.72	153.53±5.16	0.000*
Weight (kg)	73.39±10.25	67.51±12.26	0.114
Body fat (%)p	22.71±7.15	34.00±7.27	0.000*
Systolic blood pressure (mmHg)	125.00(14.20)	111.05(22.00)	0.070
Diastolic blood pressure (mmHg)	81.70±7.70	80.30±12.10	0.678
Blood glucose (mmol/L)	5.50(1.40)	5.80(2.80)	0.165
Hemoglobin (g/dL)	15.50±2.50	12.90±1.20	0.000*
Total cholesterol (mmol/L)	6.24±0.66	6.28±0.73	0.851
Triglyceride (mmol/L)	1.50±0.55	1.25±0.95	0.160
HDL-c (mmol/L)	1.37±0.26	1.63±0.38	0.022*
LDL-c (mmol/L)	4.10±0.77	3.90±0.70	0.399

*Data are presented in mean ±SD or median (IQR), $p<0.05$ indicates significant difference by independent t-test

Table 3: Effect of supplementation on blood pressure, hemoglobin and lipid profile

Parameter	**Male (n=17)**		**P**	**Female (n=23)**		**P**
	Before	After		Before	After	
Systolic (mmHg)	*125.00 (22.00)*	*114.00 (22.00)*	0.079	*111.50 (22.00)*	*110.50 (19.00)*	0.002*
Diastolic (mmHg)	81.65±7.72	73.18±7.71	0.117	80.25±12.10	78.04±11.89	0.223
Glucose (mmol/L)	*5.50(1.40)*	*5.30(0.40)*	0.008*	*5.80(2.80)*	*5.2(0.9)*	0.003*
Hemoglobin (g/dL)	15.50±2.50	15.40±1.90	0.856	12.9±1.2	13.7±1.56	0.007*
Total cholesterol (mmol/L)	6.24±0.66	5.98±0.69	0.030*	6.28±0.73	6.08±0.80	0.039*
Triglyceride (mmol/L)	*1.50(0.55)*	*1.40(0.55)*	*0.418*	*1.25(0.95)*	*1.30(1.43)*	0.396
HDL-c (mmol/L)	1.37±0.26	1.38±0.23	0.921	*1.54(0.53)*	*1.46(0.64)*	0.361
LDL-c (mmol/L)	4.10±0.77	3.69±0.71	0.060	3.90±0.70	3.74±0.74	0.284

*Data are presented in mean ±SD or median (IQR), $p<0.05$ indicates significant difference by independent t-test

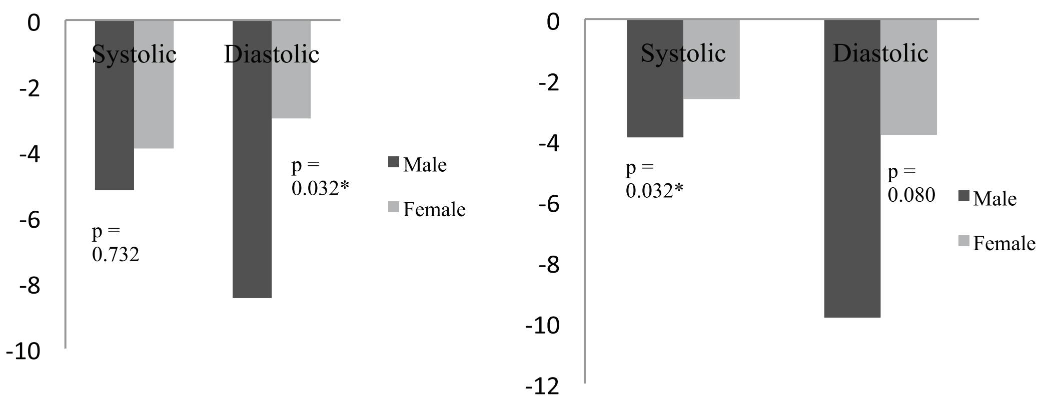

Figure 2: Absolute (in mmHg) and percent change for blood pressure

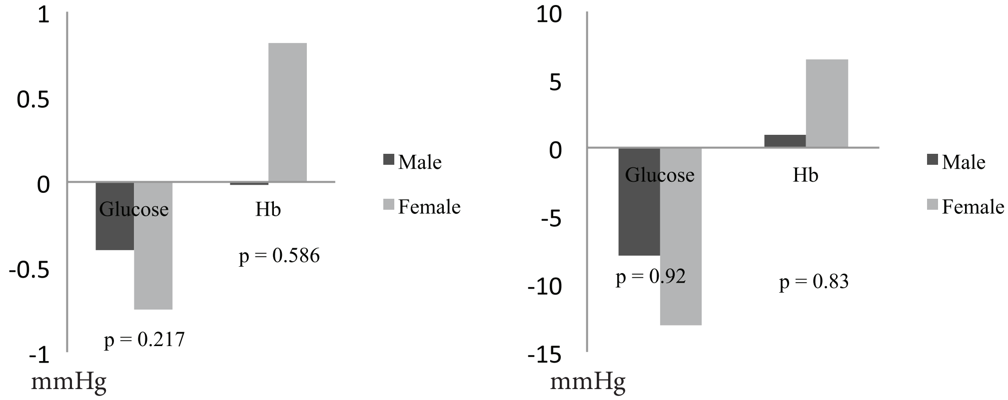

Figure 3: Absolute and percent change for blood glucose and hemoglobin

further show that there was a significant difference in absolute change for diastolic blood pressure between male and female subjects (-8.47 mmHg vs -3.00 mmHg). Therefore, eventhough there was no effect of supplementation in male subjects, based on absolute change, a greater reduction in diastolic blood pressure in male as compared to females was observed (Figure 1). Again, in terms of percent change, both systolic blood pressure (-3.9% vs +2.6%, p=0.032) and diastolic blood pressure (p>0.05) decreased at a greater value in male respondents. Geleijnse *et al*. (2002) reported that hypertensive subjects had higher response to blood pressure lowering treatment. In the present study, it was found that the systolic blood pressure for male respondents was higher (125 mmHg) as compared to females (111.5 mmHg). It has been reported that the hypotensive action of *N. sativa* is mainly caused by its volatile oils (Khosh & Khosh, 2001).

Effect of Black Seed and Honey Mixture on Blood Glucose and Hemoglobin

Table 3 shows the result of blood glucose and hemoglobin concentration at baseline and after forty-five days supplementation in male and female respondents. Figure 3 shows the absolute and percent change in blood glucose (mmol/L) and hemoglobin (g/dL) concentration between group i.e. male vs female. The present study found that the median blood glucose concentration in both male (5.50 to 5.30 mmol/L, p=0.008) and female (5.80 to 5.20 mmol/L, p=0.003) decreased significantly.

However, in terms of mean hemoglobin concentration, only females showed significant increase from 12.90 to 13.70 g/dL (p=0.007). No effect of supplementation towards hemoglobin concentration in male subjects. This could be due to higher baseline value for male as compared to female respondent, thus no further increases is needed. Previous studies reported no effect of supplementation of blackseed and honey mixture on blood glucose and hemoglobin concentrations (Yusof *et al*., 2012; Mohamad *et al*., 2014). However, the present study showed significant blood glucuse lowering effect in male and female subjects by 7.9% and 13%, respectively (Figure 3). Again, female show greater reduction in blood glucose lowering as compared to males. This could be due to higher value for blood glucose concentration at baseline in females (median: 5.80 mmol/L) as compared to males (median: 5.50 mmol/L). Sarina (2013), also reported a significant blood glucose reduction in female group. The positive effect of supplementation on blood glucose cpncentration might due to the fact that *N. sativa* seed's fixed and essential oils are rich source of phytochemicals which has been reported to prevent lifestyle disorders including hyperglycemia and hypercholesterolemia (Sultan *et al.,* 2009). The present study also show significant increase (p=0.007) in hemoglobin concentration by 6.5% in female respondents. To date, limited studies are available in explaining the faveorable effects of supplementation on hematologic parameters, but Al-Waili *et al.* (2006) found that natural honey (80g/day) decreased prostaglandin level, increased nitric oxide and thus improved hematological values among patients with long history of AIDS after three weeks of treatment.

Effect of Black Seed and Honey Mixture on Lipid Profile

Figure 4 shows the absolute and percent change for lipid profile between gender.

Table 3 shows the lipid profile data in male and female subjects at baseline and after forty-five days supplementation period. The normal value for total cholesterol is less than 5.2 mmol/L, LDL-c is less than 2.6 mmol/L, triglyceride is less than 1.7 mmol/L and HDL-c is more than 1.1 mmol/L (National Cholesterol Education Program, 2001). Based on these standard values for lipid profile, values for total cholesterol and LDL-c were not in normal range. High level of cholesterol in blood is a major modifiable risk factors for CVD (Lakoumentas *et al.,* 2005; Ji*et al.,* 2011). The results revealed that only total cholesterol decreased significantly in both male (6.24 to 5.98 mmol/L, p=0.030) and female (6.28 to 6.08 mmol/L, p=0.039) subjects by paired t-test. Yusof *et al.* (2012) and Mohamad

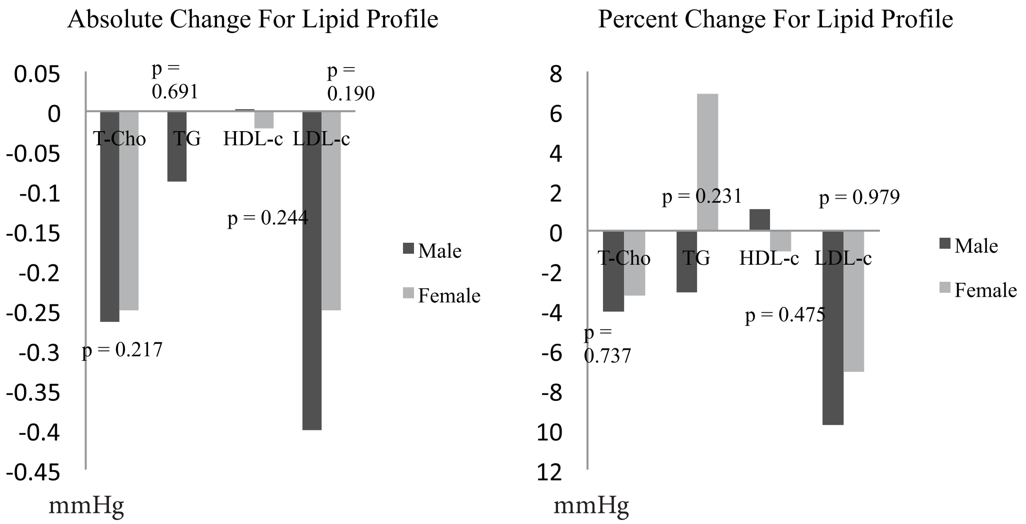

Figure 4: Absolute (in mmol/l) and percent change for lipid profile

et al. (2014) reported significant reduction in total cholesterol and also triglyceride following a 2-3 month supplementation in healthy (2.8 g/day; two months) and high cholesterol subjects (3.6 g/day; three months).

The present study show significant reduction (p<0.05) in total cholesterol by 4% and 3.3% in male and female subjects. Longer supplementation period might be needed to exhibit the beneficial effect of blackseed and honey mixture on lipid profile. In terms of gender differences, males show more favorable effect than female in lipid profile improvement (total cholesterol: -4% vs -3.3%; triglycerides: -3.1% vs +6.9%; HDL-c: +1.09% vs -1.03%; LDL: -9.8% vs -7.1%). Mohamad *et al.* (2014) also reported similar trend with significant effect on total cholesterol and HDL-c in female subjects. It could be postulated that the effect might due to the presence of thymoquinone, sterols, flavanoids, soluble fiber and polyunsaturated fatty acids (Gali-Muhtasib *et al.,* 2006; Butt & Sultan, 2010; Lutterodt *et al.,* 2010) in combination action have shown to reduce plasma cholesterol. These components also beneficial in regulating the blood lipid profile to prevent the heart vascular disease (Tulukcu, 2011). The action of *N. sativa* seeds in lipid lowering might also due to modulation of HMG-CoA reductase activity, increased levels of LDL-c receptors and by preventing LDL-c oxidation throught anti-oxidant mechanism (Ahmad & Beg, 2013). In addition, it is possible to reduce cholesterol level via intestinal cholesterol absorption which inhibited by plant sterols (Trautwein *et al.,* 2003). While fructooligosaccarides (FOS) in honey might inhibit the synthesis of fatty acids in the liver, would result in cholesterol reduction instead (Nemoseck *et al.,* 2011). It has been reported that reduction in total cholesterol reduced the incidence of cardiac events and high level in HDL-c is associated with CVD protection due to anti-inflammatory and antioxidant activities (McGrowder *et al.,* 2011). Furthermore, LDL-c levels reduction can decrease the incidence of CHD up to one third (Assmann & Gotto, 2004).

Lipid profile refers to a pattern of lipids in the blood which consists of total cholesterol, triglyceride, HDL-c and LDL-c. Excess lipid in blood (hyperlipidemia) leads to the development of coronary heart disease (CHD) (Jain *et al*., 2007). It is well known that hypercholesterolemia is due to rise in LDL-c or dyslipidemia and low in HDL-c are major risk factors that contribute to the cardiovascular diseases (CVDs). Thus, by modulating the lipid profile is an important way to prevent the CVD occurance. It was demonstrated that reduction in total cholesterol reduced the incidence of cardiac events and high level in HDL-c is associated with CVD protection due to anti-inflammatory and antioxidant activities (McGrowder *et al*., 2010). It has been reported that LDL-c levels reduction can decrease the incidence of CHD up to one third (Assmann & Gotto, 2004) and the extreme level of triglycerides can cause CHD (Koski, 2008).

Based on meta analysis, it was found that for every 10% reduction in total cholesterol, there was a 10 to 11% decrease in the risk for all cause mortality and a 13 to 15% decrease in the risk for CHD related mortality (Gould *et al*., 1998). Therefore, based on the finding of the present study, the reduction of TC by 4% can lead to some extent of CHD incidence modulation. The discussions of gender differences in reductions of CVD in lipid-lowering drugs is still generated much debate and confusion over the years (Brown *et al*., 2015). Previously, it has been concluded that gender differences in lipid profile is not apparent (Leosdottir *et al.,* 2011; Wang & Wang, 2013). However, effects of treatment based on gender is still in further exploration and the present study can contribute to a documented data that gender might play an important role in CVD risk modulation.The present study has not measured any variable in relation to their dietary intake. This is because human are hard to be controlled since they are in their normal life, not in quarantin setting. It is then anticipated that any changes occurred are due to supplementation *per se* without additional effect of dietary modification. In CVD risk modulation, there are many uncontrolled factors involved such as stress, internal health condition, health perception and beliefs, motivation, family support, environment and etc that can be of study limitation.

Conclusion

Overall, there is a significant gender differences on the effect of black seeds and honey mixture supplementation. Females showed favorable significant effects on hemoglobin, systolic blood pressure, blood glucose and total cholesterol. On the other hand, males only showed favorable significant effect on hemoglobin, blood glucose and total cholesterol. However, in terms of lipid profile, males tended to benefit more following supplementation as compared to their female counterparts. Thus, the dose of 2.3 g/day for 45 days is not sufficient to demonstrate its maximum benefits on CVD risk factor.

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