Antioxidant potential and low-density lipoprotein cholesterol (LDL-c) uptake of the black seed and honey mixture on human hepatocellular carcinoma (HepG2) cells

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Abstract

High lipid levels especially low-density-lipoprotein cholesterol (LDL-c) are associated with increased risk of cardiovascular (CVD) and coronary heart disease (CHD). Both black seed (*Nigella sativa* L.) and honey are well-known in the hypolipidemic potential and have CVD protective effects. In the present study, LDL-c uptake of the black seed and honey mixture was tested on HepG2 cells. Antioxidant activities of black seed and honey mixtures were determined through the 2, 2'-diphenyl-1-picrylhydrazyl (DPPH) assay. The anticancer potential of black seed and honey mixtures in HepG2 cells was performed using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) cytotoxicity assay. Black seed possessed the highest antioxidant activities with EC_{50} 6.54 mg/mL as compared to honey with EC_{50} value 9.56 mg/mL while the black seed and honey mixture have EC_{50} between black seed and honey. From the results obtained, no synergistic effect was observed in the mixtures as the EC_{50} values were within the range of black seed and honey. Furthermore, no significant difference ($p > 0.05$) among ratios (1:1, 2:1 and 1:2). However, the decrease in cell proliferation was the highest in black seed and honey mixture at 1:1 ratio ($p < 0.05$) than individually treated black seed and honey. Thus, the black seed and honey mixture at ratio 1:1 was the most potent anticancer agent with an IC_{50} value of 7.44 µg/mL. The present study illustrated that black seed and honey mixtures possess a lipid-lowering effect via LDL-c uptake in HepG2 cells ($p < 0.05$). The highest LDL-c uptake was observed at 15 µg/mL with the treatment of black seed and honey mixture at 1:2 ratio which was 294.4%. Further studies should be conducted on primary human liver cells to further justify the correlation between the antioxidant level and LDL-c uptake mechanism of black seed and honey mixtures.

1. Introduction

Hyperlipidaemia is a condition characterized by an abnormal elevation in one or more of the serum lipids such as total cholesterol, low-density lipoprotein cholesterol (LDL-c) and triglycerides (Shinde *et al*., 2013). Hypercholesterolemia is the main risk factor for cardiovascular diseases such as ischaemic heart disease and ischemic stroke. Elevated LDL-c is a risk factor for the development of cardiovascular disease. Recently, the role of antioxidants in plant-derived foods were studied in relation to human health. A previous study reported the beneficial effect of fruits and vegetables rich diets with a protective effect against chronic diseases including CVD (Wang *et al*., 2014) due to the medicinal compound in the plant (Saman *et al.,* 2010).

There are several forms of cholesterol-lowering drugs used to treat hypercholesterolemia. These drugs can be associated with side effects. One of the most popular cholesterol-lowering drugs, statin, has been shown to contribute to symptoms of intolerance or even adverse side effects such as myopathy, hepatotoxicity, neurological effects and diabetes mellitus (Wilkinson *et*

al., 2014). In addition, cancer chemotherapy is associated with fatigue, pain, diarrhoea, nausea and vomiting, sores in the mouth and throat, loss of appetite, hair loss and several others (Lorusso *et al.,* 2017). As a result, many people today prefer natural herbs or plants to replace their medication since herbal therapies have fewer side effects as compared to prescription drugs.

Previous studies reported that both black seed and honey are effective in lowering the serum LDL-c, possess high antioxidant activity and are effective against cancer cells (Majid *et al.*, 2013; Shafiq *et al.*, 2014; Sahebkar *et al.*, 2016). *Nigella sativa*, from the family Ranunculaceae, which is also known as black seed consists of several bioactive compounds such as thymoquinone, α-hederin, alkaloids, flavonoids, antioxidants, fatty acids and many others (Sultan *et al.*, 2015). There is evidence that shows that consumption of black seed can significantly decrease LDL-c in the blood (Asgary *et al.*, 2013; Sahebkar *et al.*, 2016) apart from total cholesterol and triglycerides lowering. Recently, Rashidmayvan *et al.* (2019) reported that *N.sativa* seed oil supplements exhibited benefits on serum liver enzymes and lipid profile in non-alcoholic fatty liver disease (NAFLD) patients. The hypocholesterolemic effect of black seed is due to the presence of bioactive compounds that can help to lower serum cholesterol such as phytochemicals, antioxidants and unsaturated fatty acids (Sahebkar *et al.*, 2016) including monounsaturated oleic acid, polyunsaturated omega-6 linoleic acid and omega-3 linolenic acid. Besides, the major bioactive compound thymoquinone and its dimer dithymoquinone, thymohydroquinone, and thymol in black seed also have an anti-cancer effect (Shafiq *et al.*, 2014). These compounds possess cytotoxicity activity against a few human cancer cell lines such as HepG2 cells, and also colon, breast and lung cancers (Shafiq *et al.*, 2014). Honey, on the other hand, has beneficial therapeutic components that are also related to its antioxidant properties. Polyphenol compounds such as phenolic acid and flavonoids, rather than enzymes (peroxidase, catalase), vitamins and mineral trace elements are known to be responsible for this action (Gheldof *et al.*, 2002).

Studies proved that consumption of honey can reduce LDL-c in serum (Majid *et al.*, 2013; Wan Ghazali and Mohamed, 2015). Extensive studies have shown that honey is effective against cancer cells including HepG2 cells. A study reported that honey exhibits its cytotoxic effects against HepG2 cells through reduction of expression of the B-cell lymphoma 2 (Bcl-2) gene which leads to apoptosis (Sadeghi-Aliabadi *et al.*, 2015). The anti-cancer properties of honey are contributed by its flavonoids and phenolic content (Erejuwa *et al.*, 2014).

One of the functions of the liver is to maintain an individual cholesterol balance by controlling the concentration of LDL-c in the plasma. The concentration of cholesterol can affect the function of the low-density lipoprotein receptor (LDLR) in the liver. LDLR is responsible for mediating the intake of LDL plasma by endocytosis regulated by the receptor and cholesterol in cells used (Goldstein and Brown, 2009). Cancer refers to the uncontrolled growth of cells that display malignant conduct. The abnormal cells expand quickly outside their natural limits and then spread to neighbouring body parts and other organs. Some of the common death from cancer are lung, liver, stomach, colorectal, breast and oesophagus (Stewart and Wild, 2015). Hepatocellular carcinoma (HCC) is also known as primary hepatic cancer. It's one of the world's most common cancer. Persistent Hepatitis B (HBV) or Hepatitis C (HCV) infections are the major risk factors for HCC. Other risk factors include aflatoxin exposure from Aspergillus fungi, alcohol, smoking, overweight, diabetes, family factors as well as dietary factors such as high intake of meat and high glycemic load (Bosetti *et al.*, 2004). HepG2 is one of the common HCC forms.

Since previous studies reported that both black seed and honey are effective in lowering the serum LDL-c, possess high antioxidant activity and are effective against cancer cells, it is, therefore, important to discover and develop a natural product from foods that has multiple effects in cholesterol-lowering through the increase of LDL-c uptake in liver and antioxidant activities. However, the exact mechanisms responsible for cholesterol-lowering are not established because there are a few mechanisms responsible for cholesterol-lowering such as LDL-c uptake, reduction of LDL-c synthesis and reduction of LDL-c absorption. In addition, several studies have documented that the combination of black seed and honey may improve health problems such as functional dyspepsia and asthma (Mohtashami *et al.*, 2015; Koshak *et al.*, 2017). Furthermore, according to Hassan *et al.* (2012), both black seed and honey are effective against HepG2 cells but further studies should be performed on the impact of their mixtures on HepG2 cells. Besides, HepG2 cells are chosen in this study because they still exhibit multiple hepatic roles, including those linked to cholesterol and triglyceride metabolism (Donato *et al.*, 2015). Thus, in the present study, the black seed and honey mixtures will be used to determine their effects on lowering LDL-c, antioxidant activity and its anti-cancer properties.

2. Materials and methods

2.1 Samples

Human Hepatocellular Carcinoma (HepG2) cells

were obtained from the Institute of Marine Biotechnology (IMB) Laboratory, Universiti Malaysia Terengganu (UMT). Eagle's minimal essential medium (EMEM) supplement and lipid-depleted fetal bovine serum (FBS) was from ThermoFisher Scientific. Trypan blue stain (0.4%) for the usage together with a hemocytometer, trypsin-EDTA solution, cholesterol (>99%), FBS, streptomycin/penstrip, phosphate buffer solution, thiazolyl blue tetrazolium bromide (MTT) were all from Sigma Aldrich, Unites States of America. 25-hydroxycholesterol was from Cayman Chemical, US and BODIPY® FL LDL was from Invitrogen, US.

2.2 Assay of 2, 2'-diphenyl-1-picrylhydrazyl (DPPH)

Antioxidant activity was evaluated using 2, 2'-diphenyl-1-picrylhydrazyl (DPPH) as described by Ramli *et al*. (2008), with some modifications. DPPH stock solution was prepared by dissolving 2.37 mg of DPPH powder in 100 mL absolute (100%) methanol. Black seed and honey mixtures stock solution were prepared with dimethyl sulfoxide (DMSO) to the concentration of 10 mM, respectively together with quercetin as the positive control. The solutions were loaded into a 96-well plate with performed serial dilution. Negative control was purely DMSO without test material. DPPH stock solution of 200 µL was applied to each treated well and incubated for 30 minutes in dark. The absorbance of the mixture was read at 517 nm using an ELISA microplate reader. The percentage of the DPPH free radical activity was calculated using the following equation:

$$\text{DPPH scavenging effect (\%)} = [(A_0 - A_1) / A_0] \times 100$$

Where A_0 was the absorbance of the control and A_1 was the absorbance in the presence of the extracts. The EC_{50} (concentration providing 50% inhibition) values were calculated using the dose inhibition curve in the linear range by plotting the extract concentration versus the corresponding scavenging effect.

2.3 Cell viability study

Cell viability was examined using MTT cell assay. The cell density of 1.0×10^4 per well was seeded in a 96 well plate and grown to confluence. Ratio concentrations of black seed and honey mixture 1:0, 0:1, 1:1, 2:1 and 1:2 were prepared and administered to cells. Cells were incubated for 16 hrs at 37^oC with 5% CO_2.

2.4 Low-density lipoprotein- cholesterol intake (LDL-c)

Confluent cultures were seeded after applying trypsin for 4 mins. Cells were then seeded at 1.0×10^4 cells per well per 100 µL culture medium in 96 well plates and allowed to grow until confluent. To study the

effect of black seed and honey mixture on the LDL-c uptake in HepG2 cells, HepG2 cells were treated with EMEM complete medium, 0.05% lipid depleted FBS, 10 µg/mL cholesterol, 1 µg/mL 25-hydroxycholesterol with different ratios of black seed: honey (1:0, 0:1, 1:1, 1:2, 2:1).

To study the effect of black seed and honey mixture combinations at different ratios of (1:0, 0:1, 1:1, 2:1, 1:2) and incubated for 16 hrs. All groups of cells were in quadruplets. Varioskan fluorescent plate reader was used to read the plate at 490 and 515 nm (Ishikawa *et al*., 2015).

2.5 Statistical analysis

Data were expressed as mean and standard deviation, of multiple determinations performed using black seed and honey mixtures derived from different concentrations with Minitab software version 14.0. One-way ANOVA was conducted and multiple comparison analysis was carried out using Fisher's LSD test. In all analyses, if $p < 0.05$, the difference was considered significant.

3. Results and discussion

In the present study, the antioxidant of black seed and honey extracts mixtures with different ratios were performed to determine the best free scavenging activity through DPPH reduction. Table 1 shows the trends indicated with the increased sample concentration subsequently increased the scavenging effects on DPPH radical. The efficiency concentration of black seed and honey mixtures to inhibit 50% DPPH radical (EC_{50}) is shown in Table 2. The EC_{50} is a parameter used to quantify antioxidant activity, with a lower value suggesting higher antioxidant potency. Among the treatments, the sample with black seed extract showed the highest DPPH reduction percentage while the sample with honey extract alone showed the lowest DPPH reduction percentage (Table 1) as shown with their EC_{50} value in Table 2. Thus, it can be observed that the antioxidant properties in the mixtures were mostly due to the antioxidant activity in black seed extract and can be suggested that there might be no synergistic effect between the black seed extract and honey extract samples.

Quercetin is a polyphenolic flavonoid compound that is commonly present in plants and plant food resources. It is a good natural antioxidant because of its ability to scavenge free radicals and bind transition metal ions (Baghel *et al*., 2012). Thus, in the present study, quercetin was selected as a positive control for the antioxidant assay. Mohamad *et al*. (2014) reported that

Table 1. DPPH Inhibition by black seed and honey mixture

Concentration (mg/mL)	% of DPPH Reduction					
	B	H	BH	BBH	BHH	Q
0	0	0	0	0	0	0
0.156	2.19(0.69)[c]	1.15 (0.52)[c]	1.31(0.27)[c]	7.32(1.00)[b]	2.56(1.02)[c]	15.25(4.59)[a]
0.313	4.15 (0.84)[c]	2.09(0.28)[c]	3.47(1.43)[c]	7.71(0.60)[b]	7.02(0.81)[b]	30.05(1.66)[a]
0.625	13.43(4.29)[b]	4.39 (0.36)[c]	6.75(1.66)[c]	15.79(1.90)[b]	9.04(1.02)[bc]	68.62(8.22)[a]
1.25	16.00(3.83)[c]	8.10 (0.54)[d]	15.97(3.04)[c]	21.37(2.65)[b]	12.53(1.40)[c]	96.81(0.61)[a]
2.5	34.24(5.06)[b]	12.42(1.42)[d]	23.75(4.83)[c]	25.91(3.04)[c]	14.13(1.35)[d]	97.93(0.56)[a]
5	40.25(1.85)[c]	28.68(2.00)[e]	38.80(2.77)[c]	45.04(5.10)[b]	31.31(4.29)[d]	97.98(0.58)[a]
10	72.13(3.65)[b]	51.38(1.87)[d]	57.02(2.97)[c]	59.28(1.87)[c]	56.79(3.16)[c]	98.42(0.48)[a]

Values are presented as mean(SD) from quadruplicate. Values with different superscript within the same row indicate significant difference ($p < 0.05$) by one-way ANOVA.
B = Black seed, H = Honey, BH = mixture of black seed and honey with the ratio 1:1, BBH = mixture of black seed and honey with the ratio 2:1, BHH = mixture of black seed and honey with the ratio 1:2, Q = Quercetin

the mixture of black seed and honey exhibited the highest lipid-soluble antioxidant capacity as compared to either black seed or honey alone. This is in contrast with the result of the present finding. However, there are probabilities where a higher concentration of black seed and honey mixtures show a synergistic effect with a higher percentage of DPPH reduction. The EC_{50} value for the honey sample extract of the present study was 9.56 mg/mL which falls in the range of EC_{50} value of other studies whereby the Indian honey samples had the IC_{50} values of antioxidant ranging between 4.5 to 66.1 mg/mL (Reshma *et al.*, 2016). EC_{50} of black seed extract in the present study was lower as compared to the previous study. A previous study reported that black seed extract with different types of extraction methods showed IC_{50} in the range of 1.07 to 4.48 mg/mL for DPPH antioxidant assay (Meziti *et al.*, 2012). Chloroform extract and ethyl acetate extract showed the best radical scavenging activity. The scavenging is most likely due to their high phenolic and flavonoid content such as carvacrol, t-antherol, 4-terpineol and thymoquinone (Meziti *et al.*, 2012).

Table 2. EC_{50} of black seed and honey mixtures

Treatment	EC_{50} (mg/mL)
B	6.54
H	9.56
BH	7.97
BBH	7.34
BHH	8.67
Q	0.76

B = Black seed, H = Honey, BH = mixture of black seed and honey with the ratio 1:1, BBH = mixture of black seed and honey with the ratio 2:1, BHH = mixture of black seed and honey with the ratio 1:2, Q = Quercetin

Lower antioxidant activity can be seen in the present study due to the aqueous extraction of black seed used in this study, which may lead to lower phenolic and flavonoid content. Besides, Islam *et al.* (2012) reported

that black seed extract had an IC_{50} value of 0.16 to 1.03 mg/mL in DPPH radical scavenging activity using different extraction methods, including essential oil, methanol extract, hexane extract, chloroform extract and ethyl acetate extract. The black seed essential oil displayed the lowest IC_{50} value at 12.76 μg/mL compared to another method of extraction. This is because 24 constituents have been identified in the black seed oil, although only 16 components have been identified in the extracts. It was confirmed that the black seed oil had the highest and the strongest radical scavenging activity compared to the extracts. Meanwhile, Haron *et al.* (2014) determined the antioxidant activity of black seeds of different origins by DPPH inhibition. They reported that black seed from Iran had the lowest IC_{50} of 1.49 mg/mL followed by black seed from Kelantan with IC_{50} of 1.79 mg/mL. The IC_{50} obtained in the present study was higher than the previous research suggesting a lower antioxidant activity in the samples used. Differences may be due to the method of extraction used in which the black seed was extracted by aqueous extraction in the present study, which may lead to lower phenolic and flavonoid contents of the extract, whereas the methanol extract and black seed oil were used in the previous study. The difference in antioxidant activity values may also be attributable to the different origins of the black seed. As shown in the previous report, local black seed (Kelantan black seed) has lower antioxidant activity than foreign black seed (Iran) as a proportion of its total phenolic content. The extraction method can also affect the antioxidant activity by which oil extraction has the highest antioxidant activity compared to other extraction solvents (chloroform and ethyl acetate) whereas the aqueous extract showed the lowest (Haron *et al.*, 2014). The low antioxidant activity of the aqueous black seed extract was also proven in the present study.

Based on Table 3, it was observed that all ratios

Table 3. Cell viability of black seed and honey mixtures at different ratios

Concentration (mg/mL)	Percentage of Cells Viability (%)				
	B	H	BH	BBH	BHH
0	100	100	100	100	100
1.562	65.27(5.43)[b]	76.37(0.22)[a]	64.92(1.07)[b]	80.63(0.49)[a]	66.93(0.41)[b]
3.125	65.97(8.67)[a]	72.26(2.36)[a]	63.88(0.81)[b]	74.57(2.20)[a]	65.68(0.33)[b]
6.25	63.06(7.29)[ab]	67.64(2.14)[a]	57.77(0.11)[b]	64.98(3.69)[a]	60.32(2.17)[ab]
12.5	46.55(3.45)[b]	65.54(5.09)[a]	40.44(5.23)[c]	45.68(2.23)[c]	53.46(6.12)[b]
25	19.23(3.57)[d]	52.82(0.31)[a]	31.78(3.01)[c]	37.72(0.48)[b]	36.94(4.30)[b]
50	6.12(0.66)[d]	41.75(6.31)[a]	6.51(0.42)[d]	19.22(4.92)[c]	35.10(1.05)[b]
100	5.15(0.13)[c]	37.80(1.27)[a]	3.45(0.10)[d]	5.57(0.11)[c]	32.87(0.33)[b]

Values are presented as mean(SD) from quadruplicate. Values with different superscript within the same row indicate significant difference ($p < 0.05$) by one-way ANOVA.
B = Black seed, H = Honey, BH = mixture of black seed and honey with the ratio 1:1, BBH = mixture of black seed and honey with the ratio 2:1, BHH = mixture of black seed and honey with the ratio 1:2.

lowered the HepG2 cell viability on a dose-dependent pattern. Among all treatments, the mixture of black seed and honey with a 1:1 ratio showed the significantly ($p < 0.05$) lowest percentage of cell viability (3.45%) at the highest concentration, 100 µg/mL. The lowest cell viability might be due to the synergistic effect of both black seed extract and honey extract at 1:1 ratio, but not at 2:1 or 1:2 ratio mixture. However, the effect of the black seed and honey mixture of 1:1 ratio was most probably contributed by the potential of black seed which was shown by the lowest percentage of cell viability of black seed extract at 50 µg/mL.

Table 4 shows that black seed extracts possessed the most potent anticancer activity against HepG2 cells (IC_{50} value 7.62 µg/mL) while honey extracts possessed the lowest IC_{50} value 31.10 µg/mL. All treatments were ideal anti-cancer agents with IC_{50} values of less than 20 µg/mL except for the treatment with honey extract alone with the moderate cytotoxic activity of IC_{50} values between 20 to 100 µg/mL (Sajjadi *et al.*, 2015). In general, the anticancer effect of black seed and honey mixtures could be due to the anti-cancer properties of black seed instead of honey, particularly at a lower concentration. In general, the anticancer effect of black seed and honey mixtures could be due to the anti-cancer properties of black seed instead of honey, particularly at lower concentrations. However, there was a possibility that black seed and honey mixture 1:1 ratio to show the synergistic effect at a higher concentration since the mixture showed a significant reduction of cell viability starting from 12.5 µg/mL.

According to Hassan *et al.* (2012), treatment of HepG2 cells with the black seed at the highest concentration of 5000 µg/mL reduced the survival rate of HepG2 cells by 37.8% after 72 hrs of treatment which had a lower effect as compared to the present study. In

the present study, black seed showed a higher reduction of cell viability at a lower concentration (100 µg/mL) of extracts and a shorter treatment period (24 hrs). This is due to the different types of black seed as the black seed used in the previous study was from Egypt. For honey, a previous study showed that Gelam honey exerted high cytotoxic on HepG2 cells and human hepatic cells (WRL-68). Gelam honey reduced the cell viability of HepG2 less effectively by showing IC_{50} at 25% w/v of 31.10 µg/mL which is equivalent to 0.0031% w/v. This proved that Gelam honey exhibited growth inhibition at higher concentrations and was less sensitive in HepG2 cells as compared to the current honey sample.

Table 4. Summary of IC_{50} value of black seed and honey mixtures against HepG2 Cells

Treatment	IC_{50} (µg/mL)
B (Black Seed: Honey – 1:0)	7.62
H (Black Seed: Honey – 0:1)	31.10
BH (Black Seed: Honey – 1:1)	7.44
BBH (Black Seed: Honey – 2:1)	10.98
BHH (Black Seed: Honey – 1:2)	15.23

$IC_{50} < 20$ µg/mL = highly cytotoxicity, IC_{50} 21-200 µg/mL = moderately cytotoxicity, IC_{50} 201-500 µg/mL = weakly cytotoxicity, $IC_{50} > 501$ µg/mL = no cytotoxicity.

In the present study, black seed showed a higher antioxidant scavenging effect and cytotoxicity towards HepG2 cells as compared to honey. Although the black seed and honey mixtures did not show the potential of synergistic effect in antioxidant activity, there might be a synergistic effect on the HepG2 cells cytotoxicity. Thus, the cytotoxicity effect might not be attributable to antioxidant properties but due to other mechanisms not explored in the present study. Apart from antioxidant compounds, the other pathways that could be responsible for reducing cell proliferation on HepG2 cells were p53 protein, cell cycle arrest, antimutagenic activity, estrogen modulation, anti-inflammatory activity,

immunomodulatory activity, cyclooxygenase-2 (COX-2) modulation and tumour necrosis factor (TNF) modulation (Ahmed and Othman, 2013).

Figure 1 shows that the highest LDL uptake was exhibited by the black seed and honey mixture at ratio 1:2 with 294.4% at 15 µg/mL as compared to the positive control. This suggests that the black seed and honey mixture might gave a synergistic effect.
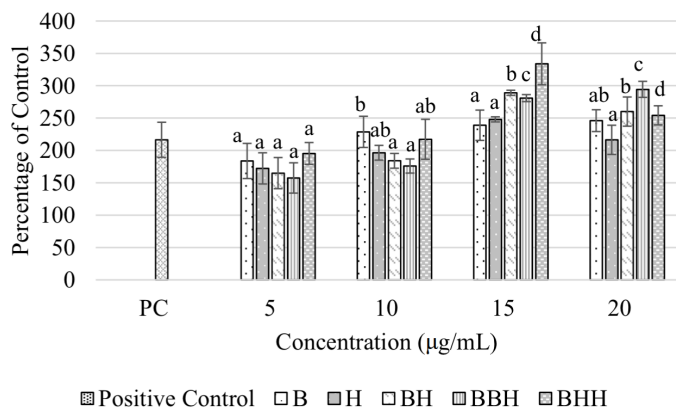

Figure 1. Effect of black seed and honey extracts mixtures on LDL-c uptake in HepG2 cells. Values are presented as mean (SD) from quadruplicate. Bars with the different notation within the same group indicate significant difference (p<0.05) by one-way ANOVA.
B = Black seed, H = Honey, BH = mixture of black seed and honey with the ratio 1:1, BBH = mixture of black seed and honey with the ratio 2:1, BHH = mixture of black seed and honey with the ratio 1:2.

A previous study showed that black seed and honey mixture had significant benefits in hypercholesterolemic subjects as compared to healthy subjects. For hypercholesterolemic subjects, their total cholesterol, triglycerides, and the ratio of TC: HDL-c decreased significantly while HDL-c increased significantly; while there was also a decrease in LDL-c but not significantly (Mohamad *et al.*, 2014). Thus, it shows that the result was correlated with the present study whereby the black seed and honey mixture showed a synergistic effect on cholesterol-lowering. El-Kholy *et al.* (2007) reported that rats with the treatment of black seed and honey mixture showed significant improvements in total lipids, total cholesterol, triglycerides, HDL-c and LDL-c as compared to the treatment of black seed and honey alone.

In the present study, black seed extract showed high LDL uptake (246.0%) at 20 µg/mL while honey extract had LDL-c uptake by 248.0% at 15 µg/mL. Previously, Ibrahim *et al.* (2014) found that menopausal women that were orally given one gram of black seed capsules daily for two months demonstrated significant improvement in total cholesterol, triglycerides, LDL-c, HDL-c and blood glucose although there were no significant changes in

body weight. On the other hand, young healthy Pakistani males who received 70 g of honey every day for 4 weeks showed a significant decrease in total cholesterol and LDL level while HDL increases significantly. However, there were no significant changes in their blood glucose and triglyceride (Majid *et al.*, 2013).

A thymoquinone-rich fraction from black seed was observed to induce LDL-c uptake through the upregulation of LDL receptor (LDLR) gene and inhibition of cholesterol synthesis through suppression of 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase (HMGCR) gene in HepG2 cells more than thymoquinone. This suggests that the effect of the thymoquinone-rich fraction may be due to the combination of rich components in black seed (Al-Naqeep *et al.*, 2009). Besides, quercetin in honey increases the clearance of circulating LDL-c levels from the blood through up-regulation of LDLR gene expression at both mRNA and protein levels in HepG2 cells (Moon *et al.*, 2012). Based on this study, it can be observed the lower antioxidant activity was due to the aqueous extraction method of black seed used which may contribute to lower phenolic and flavonoid content. However, since the LDL expression in the black seed and honey mixture was not parallel to antioxidant activity, there are possibilities that other mechanisms may contribute to LDL-c uptake in HepG2 cells. LDL-c uptake may be due to the down-regulation of proprotein convertase subtilisin/kexin type 9 (PCSK9) gene which was responsible for the degradation of LDLR in lysozyme and other lipoproteins metabolism-related gene expression includes sterol regulatory element-binding protein 2 (SREBP2) (Lagace, 2014).

4. Conclusion

The present study was the first research investigating the potential effect of black seed and honey extracts mixtures on lipid uptake in HepG2 cells. The black seed and honey mixture with a ratio of 1:2 was observed with the highest LDL-c uptake (294.4%) at 15 µg/mL. On the other hand, there was no possible synergistic effect on the antioxidant properties of the black seed and honey mixtures. This is proved by the highest EC_{50} 6.54 mg/mL in black seed extract as compared to other treatments. Since cancer cells, HepG2 cells were used in the present study for the determination of LDL-c uptake and antioxidant, the anticancer potential of black seed and honey mixtures were observed too. Black seed and honey mixtures with a ratio of 1:1 exhibited the strongest anticancer agent with an IC_{50} value of 7.44 µg/mL as compared to other treatments. In conclusion, high EC_{50} in antioxidants is not necessarily linked to high LDL-c uptake and high reduction of cell viability (IC_{50}).

Therefore, it was suggested that there may be other mechanisms that lead to LDL uptake and reduction of cancer cell viability. The present study has demonstrated evidence on the hypocholesterolemic, antioxidant and anticancer potential of black seed and honey extracts mixtures.

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