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Comparative biochemical studies of aqueous extract of Egyptian black and raw garlic (*Allium sativum*) on hypercholesterolemia in rats fed a high-fat diet

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ABSTRACT

Black garlic is a type of garlic that is processed for a specific period, with precisely controlled degrees of moisture and heat until the color of raw garlic turns black. Black garlic plays a strong role in preventing diseases such as hyperlipidemia. This study aims to compare the effect of Egyptian black garlic extract (EBG) and Egyptian raw garlic extract (ERG) at different concentrations on serum biochemical parameters in hyperlipidemia rats' model. Fifty albino rats were divided randomly into 10 groups with five animals. Rats were fed a normal diet (control) G1, high-fat diet (HFD) 40% lard w/w were (G2- G10) for four weeks to promoting hypercholesterolemia for all rats except G1 (control). After that, HFD groups were treated with 1ml oral administration three times a week of 100% (G3), 75% (G4), 50% (G5), and 25% (G6) EBG extract for 6 weeks. G2 (HFD) was a high-fat diet alone without any extract. ERG extract feed group were treated in the same manner described above, and G7 (HFD+100% ERG extract), G8 (HFD+75% ERG extract), G9 (HFD+50% ERG extract), and G10 (HFD+25% ERG extract) as written here. EBG extract-treated group showed significantly increased high-density lipoprotein cholesterol (HDL-C) in serum ($p < 0.05$), decreased both level of total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and triglycerides (TG) in serum. EBG extract with high concentration (G3) causes down regulation of lipid and cholesterol ($p < 0.05$) comparing with ERG extract indicating usefulness for treating hypercholesterolemia. Body weights on rats decrease by feeding of EBG extract, along with liver enzymes decrease in EBG fed rats. This indicates the efficiency of black garlic extract in reducing the level of hypercholesterolemia and avoiding heart disease and atherosclerosis.

Keywords: *Allium sativum*, garlic, black garlic, hypercholesterolemia, biochemical parameters.

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INTRODUCTION

High levels of blood cholesterol are one of the most important pathological problems at present because it is directly related to cardiovascular disease, being a direct relation with the high level of blood cholesterol (Matos et al., 2005).

Medical importance of garlic (*Allium sativum*) was known from ancient days in Egypt as traditional herbal medicine (Srivastava and Pathak, 2012). The most important active compounds are organosulfur compounds such as diallyl thiosulfonate (allicin), diallyl disulfide (DADS), S-allyl cysteine (SAC), diallyl sulfide (DAS), Diallyl trisulfide (DATS), and S-allyl cysteine sulfoxide (alliin), (Ushijima et al., 2018). Allicin from garlic powder had a great ability to lower serum cholesterol, triglycerides, and simultaneously the systolic blood pressure and might have a beneficial effect on atherosclerosis (Ali et al., 2000). Garlic (*Allium sativum*) and black garlic (black *Allium sativum*) have a lot of medicinal actions include antitumor and antibacterial (Sasaki et al., 2017), antihyperlipidemic, antihyperglycemic (Young et al., 2009), immune system enhancement (Wang et al., 2010), because of including biological active constituents (Azene and Mengesha, 2015). When garlic is injured either by chopping, chewing or crushing, alliin converted to allicin. Allicin contributes to the attribute flavor and taste of garlic (Kimura et al., 2017).

However discomfort smell of garlic disappear by thermal processing and improve food quality, tastes, aromas, and texture, along with formation of beneficial compounds that are not present in raw garlic (Sasaki et al., 2007; Capuano and Fogliano, 2011). Under high temperature and humidity treatment raw garlic gradually turns from white to black in color. Fresh garlic included allicin responsible for pungent odor was changed to water-soluble antioxidant compounds such as S-allyl cysteine and tetrahydrocarbolines that are bioactive alkaloids and flavonoids (Amagase, 2006).

Several studies have investigated that black garlic exerted hypocholesterolemic effect and all levels of lipids parameters in an animal model of hyperlipidemia. The total cholesterol and triglyceride were decreased by the consumption of a diet containing black garlic and improve insulin resistance. HDL-cholesterol levels were increased (Jung et al., 2011; Kim et al., 2011).

Black garlic created in Japan has been a long period consumed, together with South Korea, and other countries. Recently researchers and citizens pay attention to black garlic's biofunctions as an antioxidant, antiallergic, anti-diabetes, anti-inflammation, and anticancer (Jung et al., 2011, Yoo et al., 2014; Jing, 2020). This is because around 38 compounds in raw garlic changed into others during thermal treatment to make black garlic (Liang et. al., 2015).

In this study, we focused on chemical constituents comparison between Egyptian raw garlic (ERG) and Egyptian black garlic (EBG) first, and anti-hypercholesterolemia activity was tested by using both extracts in a rat model. as a natural bioactive plant which used over the world.

MATERIALS AND METHODS

Chemicals and reagents

Assay kits for total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), triglycerides (TG), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and creatinine were purchased from MDSS GmbH -Hannover, Germany. Other chemicals and reagents were obtained from Sigma company of the highest grade available.

Preparation of Egyptian black garlic extract (EBG) and Egyptian raw garlic extract (ERG)

Egyptian garlic was obtained from the local market in Egypt and used for black garlic processing. Briefly raw garlic bulb was incubated for 10 days at 65 to 70 °C without peeling. One hundred gram of black garlic were sliced into small pieces, followed by grinding with a mortar to produce smooth paste. Then garlic paste was solved in 100 ml of distilled water in a conical flask and filtered with a piece of gauze to obtain an aqueous solution of extract. The filtrate was stored at 4°C till use. Raw garlic extract were similarly prepared in a similar manner, grinding 100 g of raw garlic with 100 ml and filtrated with gauze to make 100% concentration of Egyptian raw garlic (ERG). The residue was weighed (10 g). The concentration of garlic extract would be equal to 90-gram garlic in 100 ml distilled water (0.9 g/1 ml). Chemical composition of moisture, ash, crude protein, crude lipids of EBG, and ERG were determined as outlined in A.O.A.C (2000).

Determination of total polyphenolic contents

The total polyphenolic content in garlic extract was estimated calorimetrically using Folin - Ciocalteau assay (Singleton et al., 1999). An aliquot of the diluted garlic extract (0.1 ml) was mixed with 0.5 ml Folin-Ciocalteau (diluted 1:10 v/v) reagent and allowed to stand for 5 min at room temperature then sodium carbonate (0.4 ml, 7.5%) was added and allowed to stand for 2 h in a dark place at room temperature. The absorbance was recorded at 750 nm by UV-Vis spectrophotometer (Shimadzu, UVmini-1240, Japan). The total polyphenolic content in the extract was calculated as milligrams equivalent of gallic acid (3,4,5-trihydroxy benzoic acid).

Animals, diets, and experimental design

Animal tests were carried out using the adult albino rats (male) weighing 140 to 165 g body weight acquired from the animal laboratory of the National Center for Radiation Research and Technology (NRRT), Egyptian Atomic Energy Authority (EAEA). Animals were housed in the experimental animals' unit under standard environmental conditions and hygiene. Rats were adapted for 7 days to the environment; thereafter, ten groups with 5 rats in each cage were made. Nine groups of rats were fed with a high fat diet, and one as control was fed with a normal diet (A.O.A.C., 2000) for four weeks before using garlic extract. High-fat diet was prepared by mixing 40% animal fat with basil diet, and oral cannulation was adopted for feeding precise dosage of extract three times a week. All experiments were carried out in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23) revised 1996 or the UK Animals (Scientific Procedures) Act 1986 and associated guidelines.

Groups were designed as follow:

Group 1 (G1): basil diet
Group 2 (G2): high-fat diet (HFD) with 40% lard w/w on the basil diet.
Group 3 (G3): HFD + 1ml EBG extract 100% three times a week.
Group 4 (G4): HFD + 1ml EBG extract 75% three times a week.
Group 5 (G5): HFD + 1ml EBG extract 50% three times a week.
Group 6 (G6): HFD + 1ml EBG extract 25% three times a week.
Group 7 (G7): HFD + 1ml ERG extract 100% three times a week.
Group 8 (G8): HFD + 1ml ERG extract 75% three times a week.
Group 9 (G9): HFD + 1ml ERG extract 50% three times a week.
Group 10 (G10): HFD + 1ml ERG extract 25% three times a week.

Serum sampling

After 6 weeks feeding of the extract, blood samples were collected from the heart, and serum was separated by centrifugation at 4000x for 10 min (Beckman model centrifuge) and stored at -20°C till use.

Biochemical assays

Serum cholesterol (TC) was determined according to the procedure described by Allain et al. (1976). High-density lipoprotein cholesterol (HDL-C) was evaluated by Grossman et al. (1976), triglycerides (TG) was described by Wadehra (1990). Aspartate aminotransferase (AST), and Alanine aminotransferase (ALT) were assayed by the method of Bergmeyer and Horder (1986a,b), and creatinine was estimated by the method of Henry et al. (1974). All evaluations were measured using enzymatic methods by commercial kits (MDSS GmbH-Hannover, Germany). Low-density lipoprotein cholesterol (LDL-C) was calculated according to the next calculation:

LDL-C = TC (HDL-C) + (TG/5), (Alex and Laverne, 1983).

Statistical analysis

One-way (ANOVA) was employed for statistic evaluation. Analysis of variance done to determine the mean difference at the level of $p < 0.05$, data results expressed as (mean ± standard deviation), (SPSS, 2009).

RESULTS

Chemical composition of Egyptian black garlic extract (EBG) and Egyptian raw garlic extract (ERG)

The chemical composition of EBG and ERG was summarized in Figure 1. The moisture content of EBG decreased significantly ($p < 0.05$) compared to that of ERG, while protein and fat significantly increased in EBG than in ERG. The total polyphenols were three times higher in EBG than in ERG. An increasing amount of polyphenol plays an important role as an antioxidant activity in EBG comparing with ERG.

Effect of EBG and ERG on body weight and food intake in rats

As shown in Figure 2, the initial body weight in G2 (high-fat diet without garlic extract) was different from EBG and ERG extract fed rats. The initial body weight of G3 (HFD+100% Egyptian black garlic extract) slightly decreased ($p < 0.05$) comparing with G2. The initial body weight of G7 (HFD+100% Egyptian raw garlic extract) was decrease than G2. No dose-dependent effect of ERG extracts was observed against body weight (G7, G8 and G10). But EBG extracts showed made reduction significantly against the initial body weight G3, G4, G5 and G6 ($p < 0.05$) six weeks later from treatment the final body weight of G3 was decreased ($p < 0.05$) against G2, but not significant between G2 and G7. Additionally, the final body weight in the HFD group was higher than that of the animals fed EBG and ERG.

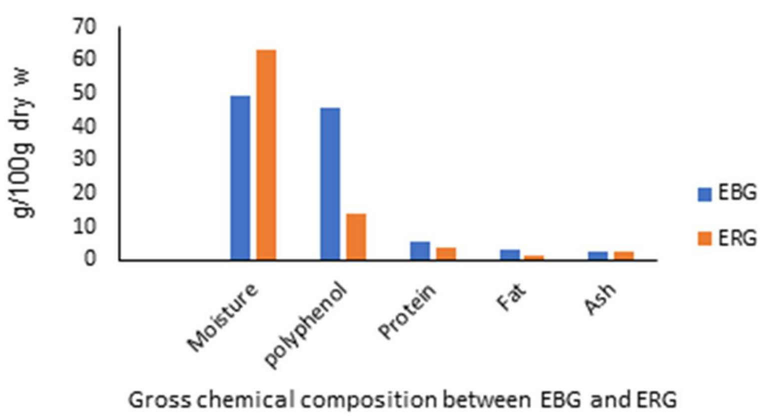

Figure 1. Compare between the chemical composition of Egyptian black garlic (EBG) and Egyptian raw garlic (ERG).

Effect of EBG and ERG to the serum lipid profile

Total cholesterol (TC)

On the effect of black (EBG) and raw garlic (ERG) extracts to serum lipid profile in rats fed a high-fat diet, EBG intake rats decreased serum total cholesterol levels compared with G2 (high-fat diet without garlic extract (Table 1). Total cholesterol in EBG extracts treated was less level than in ERG fed. Wholly EBG treated rats showed lower total cholesterol level in serum (155.9 to 226.78 mg/ dl) than those in the ERG feeding group. This finding demonstrates that the addition of Egyptian black garlic extracts minimized the rate of total cholesterol in serum.

Total triglyceride (TG)

Total triglycerides level in G3 (HFD+100% EBG) was

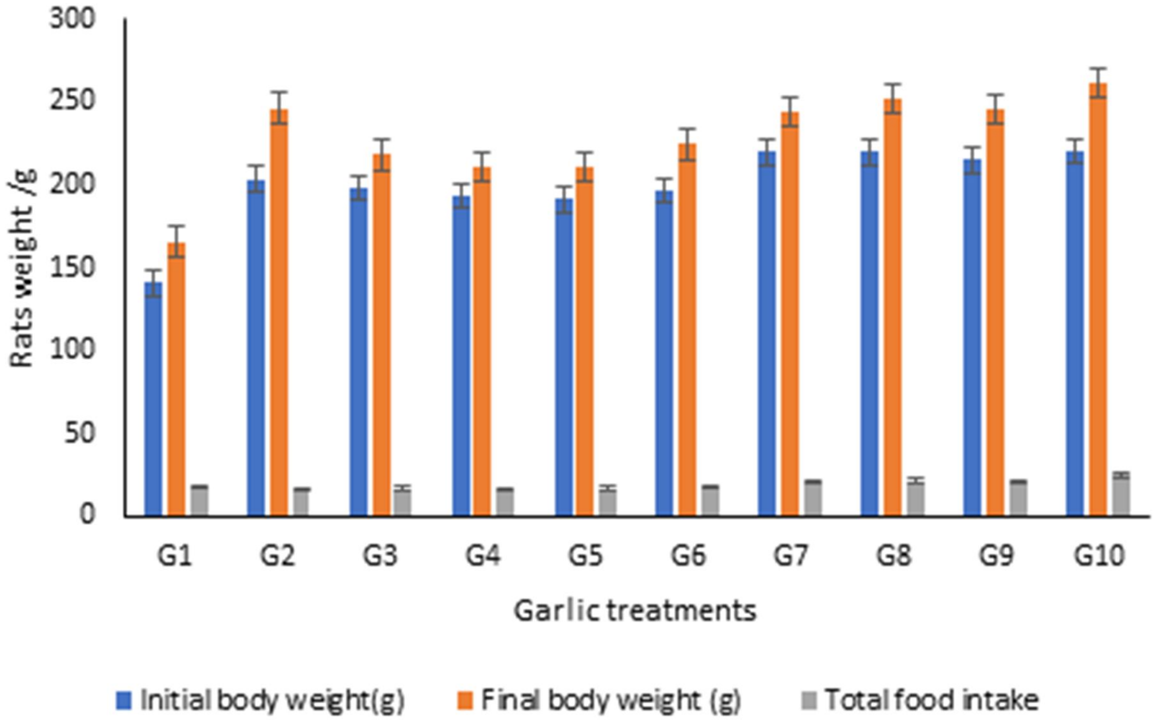

Figure 2. Graphical comparison of mean values of control group and test groups for rats' body weight and food intake. G1 (control), G2 (HFD only), G3 ((HFD+100% EBG), G4 (HFD+75% EBG), G5 (HFD+50% EBG), G6 (HFD+25% EBG), G7 (HFD+100% ERG), G8 (HFD+75% ERG), G9 (HFD+50% ERG) and G10 (HFD+25% ERG).

Table 1. Total cholesterol (TC), total triglycerides (TG), HDL-C, and LDL-C level in rats fed with different concentrations of Egyptian black garlic extract (EBG) and Egyptian raw garlic extract (ERG).

Parameter	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	*p < 0.05*
TC (mg/dl)	200.95 ± 5.39^{f}	241.5 ± 7.62^{a}	155.9 ± 4.02^{i}	177.96 ± 4.36^{h}	192.98 ± 6.86^{g}	226.78 ± 5.64^{c}	194.61 ± 3.92^{g}	211.56 ± 2.88^{e}	222.72 ± 3.93^{d}	238.64 ± 4.03^{b}	***
TG (mg/dl)	114.14 ± 3.02^{e}	185.93 ± 4.17^{a}	149.89 ± 6.37^{d}	156.62 ± 7.08^{d}	156.03 ± 7.05^{d}	163.86 ± 6.21^{cd}	177.04 ± 5.88^{abc}	167.67 ± 4.28^{bcd}	178.03 ± 4.04^{abc}	184.28 ± 6.48^{ab}	***
HDL (mg/dl)	51.84 ± 0.69^{b}	40.22 ± 0.63^{e}	52.91 ± 0.58^{a}	51.16 ± 0.47^{b}	49.43 ± 0.48^{c}	48.76 ± 0.78^{c}	48.78 ± 0.74^{c}	40.72 ± 0.68^{d}	40.72 ± 0.27^{e}	39.01 ± 0.24^{f}	***
LDL (mg/dl)	130.03 ± 4.42^{e}	165.22 ± 3.64^{a}	72.96 ± 2.53^{h}	94.95 ± 2.58^{g}	112.67 ± 3.20^{f}	142.36 ± 4.44^{c}	112.95 ± 2.77^{f}	137.31 ± 3.92^{d}	147.56 ± 5.04^{b}	164.37 ± 5.01^{a}	***

G1 (control), G2 (HFD only), G3 ((HFD+100% EBG), G4 (HFD+75% EBG), G5 (HFD+50% EBG), G6 (HFD+25% EBG), G7 (HFD+100% ERG), G8 (HFD+75% ERG), G9 (HFD+50% ERG) and G10 (HFD+25% ERG). Values are shown as mean ± standard deviation (n=5). Different superscript letters in the same line indicate a statistically significant difference for $p< 0.05$ (one-way ANOVA).
*** mean highly significant difference between all treatments.

lower than G2 (HFD without extracts), but no effect was observed in less EBG extracts, but there was no statistical significance in G3 to G6 against G2, and ERG feeding groups G7 to G10 slightly decreased against G2 (HFD).

High-density lipoprotein cholesterol (HDL-C)

HDL-C in G3- G6 (HFD+ black garlic extracts) were significantly increased in rats against the HFD group (G2 without extracts). Black garlic extracts was potential compound to reduce HDL-C level in serum and recommendable compounds to regulate cholesterol and triglycerides level in serum. The greatest value of HDL-C was in G3 (EBG 100% HFD) but the least value was in G10 (EFG 25% +HFD). All values of HDL-C were significant differences except G5, G6 and G7 were non-significant ($p < 0.05$). The animals fed on a high-fat diet enriched with Egyptian black garlic and Egyptian raw garlic evidenced a decrease in HDL-C level. But the effect of a high concentration of Egyptian black garlic was the best in lowering total HDL-C comparing with non-garlic extract and adding raw garlic extract with different concentrations.

Low- density lipoprotein cholesterol (LDL-C)

Black garlic extracts (EBG) worked to lower LDL-C levels in serum, indicating beneficial effects to maintain healthy blood vessels.

Effect of EBG and EFG to ALT, AST, and creatinine

Table 2 shows the effect of varied diet composition on the activity of ALT, AST, and creatinine. There was no significant difference among all groups with serum levels of creatinine while the activity of ALT for rats consuming the high-fat diet (G2) was slightly increased towards all treatments of EBG and EFG (38.12 U/l). AST had higher values in G2 (HFD) as compared to all treatments of EBG or ERG. The differences were slightly significant ($p < 0.05$) in all treatments

Table 2. Alanine transaminase (ALT), Aspartate transaminase (AST), and creatinine level in rats fed different concentrations of Egyptian black garlic extract (EBG) and Egyptian raw garlic extract (ERG).

Parameter	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	*p < 0.05*
ALT (U/L)	32.02 ± 0.17[f]	38.12 ± 0.08 [a]	26.45 ± 0.28 [h]	31.40 ± 0.28 [g]	32.07 ± 0.12[f]	33.32 ± 0.23[e]	34.4 ± 0.23 [d]	35.34 ± 0.10[c]	35.21 ± 0.17[c]	37.63 ± 0.18[b]	***
AST (U/L)	28.40 ± 2.16 [f]	36.19 ± 0.14 [a]	31.14 ± 0.14 [de]	32.13 ± 0.11[bcd]	33.06 ± 0.23[b]	32.34 ± 0.13[bc]	31.14 ± 0.15 [de]	31.62 ± 0.35[cd]	31.41± 0.34[cde]	30.52 ± 0.83 [de]	***
Creatinine (mg/dl)	1.1 ± 0.02[a]	1.08 ± 0.01[a]	1.04 ± 0.01 [a]	1.02 ± 0.01a[b]	1.02 ± 1.01[ab]	0.45 ± 0.02[c]	1.02 ± 0.001[ab]	0.95 ± 0.02[ab]	0.87 ± 0.02[ab]	0.80 ± 0.01[b]	***

G1 (control), G2 (HFD only), G3 ((HFD+100% EBG), G4 (HFD+75% EBG), G5 (HFD+50% EBG), G6 (HFD+25% EBG), G7 (HFD+100% ERG), G8 (HFD+75% ERG), G9 (HFD+50% ERG) and G10 (HFD+25% ERG).
Values are shown as mean ± standard deviation (n=5). Different superscript letters in the same line indicate a statistically significant difference for $p< 0.05$ (one-way ANOVA).
*** mean highly significant difference between all treatments.

DISCUSSION

Previous studies were notified that garlic supplemented diets may ban the production (synthesis) of cholesterol and fatty acids in the liver (Yeh and Liu, 2001). Egyptian black garlic (EBG) is the fermented Egyptian raw garlic (ERG) (*Allium sativum* L.) for a period of time at temperatures 65 to 70°C and high humidity for 10 days. During the thermal process caused by the Maillard reaction which is known as a non-enzymatic reaction between amino groups in amino acids and reducing sugars. 5-Hydroxy Methyl Furfural (5-HMF) and 2-acetyl pyrrole has resulted as intermediate products of the Maillard reaction. The pleasant flavor in black garlic was contributed by 2-acetyl pyrrole (Jing, 2020; Lu et al., 2017).

The fermentation process turns garlic cloves to blackish and gives them a sweet taste. This process reduces some strong pungent-flavor found in raw garlic due to reducing the content of allicin. Alliin (S-allyl cysteine sulfoxide) contained in raw garlic was digested with alliinase by garlic crushing and converts to allicin (diallylthiosulfinate). Raw garlic contains alliin (S-allyl Cysteine Sulfoxide), alliinase enzyme activated through garlic crushing, and convert alliin into allicin (diallyl thiosulfinate). Allicin unstable under high-temperature conditions and can be turned into other sulfur-containing derivatives in a short period giving black garlic a unique flavor (Qiu et al., 2020). Lately fascinating data was presented by Japanese researchers on chemical constituents of the volatile derived from black garlic processing (Hamano et al., 2019). It indicated that pungent gas released from the black garlic processing have consisted of ammonia, sulfide- and aldehyde-groups as major three compounds, probably associated with anti-bacteria activity to protect themselves from harmful enemies to survive in nature.

The gross chemical composition between EBG and ERG indicated that the moisture content of black garlic was decreased than raw garlic but crude protein and total polyphenol were increased in black garlic than in raw garlic. The value of total phenols was 13.91 mg gallic / 100 g dry weight in raw and 45.5 mg gallic/100 g in black garlic. Kim et al. (2011) suggested that phenolic compounds were increased by about four to tenfold in black garlic compared with raw garlic which plays an important role as antioxidant properties (Kim et al., 2012). Black garlic exerted hypocholesterolemic and hypoglycemic effects in animals' model of type 2 diabetes mellitus or invitro antioxidant effect on human low-density lipoprotein (Seo et al., 2009).

Also, serum level of high-density lipoprotein cholesterol (HDL-C) in a high-fat diet group (HFD) supplemented with black garlic was significantly increased in all different concentrations of EBG (G3, G4, G5 and G6) compared to those in the HFD group (G2). Black garlic improves lipid metabolism and its obesity function may be related to the decreases in expression of hepatic sterol regulatory element-binding protein which play a major mediator of insulin action on the hepatic expression of glucokinase and lipogenesis-related genes (Kimura et al., 2017; Seo et al., 2009; Ha et al., 2015; Choi et al., 2014).

Chemical compounds of BG depend on the conditions during the thermal processing. As beneficial compounds included BG were increased during the thermal processing, particularly polyphenols, flavonoids, and some of the Maillard reaction intermediates known as antioxidant agents (Kimura et al., 2017; Hwang et al., 2011). Anti-cholesterol activity was one of the major bioactivities have been reported in the past like anticancer, antibacterial, antioxidant, immune enhancement, liver function improvement (Vokk et al., 2014; Sato et al., 2006).

CONCLUSION

Administration of Egyptian black garlic extract (EBG) was effective to improve cholesterol level, triglycerides, and high-density lipoprotein in the blood serum of rats fed with a high-fat diet. A lower dosage of the black garlic extracts was less effective in affection to the lipid levels. Polyphenols amount included had increased in black garlic compared to raw garlic. Constant ingestion of black garlic in daily life is beneficial for the prevention of hyperlipidemia caused by a high-fat diet. Additionally, liver enzymes became low by treatment of black garlic extracts.

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