Hypolipidemic and antioxidant potential of bitter gourd (*Momordica charantia* L.) leaf in mice fed on a high-fat diet

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Abstract: The purpose of this study was to investigate the antioxidant and hypolipidemic potential of bitter gourd (BG) leaf ethanol extract (LE) in mice fed a high-fat diet (HFD). Fifty mice were randomly separated into five groups with 10 animals of each group. The animals received normal diet (NC), HFD diet (HF), 200mg/kg/day LE with HFD (LLE), 400 mg/kg/day LE with HFD (MLE), 800mg/kg/day LE with HFD (HLE), respectively. After six weeks, HF group showed meaningfully ($P<0.05$) increased body weight, fat index, serum lipid and oxidant stress compared to NC group. However, serum TC, TG and LDL-c concentrations were lower in all LE treated groups compared with HF group ($P<0.05$). In addition to LLE group, HLD-c levels in LE treated groups were higher that that in HF group ($P<0.05$). Moreover, LE attenuated significantly ($P<0.05$) the MDA content and elevated the SOD activities of the liver tissues in a dose effect relationship. The histopathological examination confirmed the hepatoprotective effect of LE against liver damage induced by HFD. These findings illustrate that bitter gourd leaves may be valuable for preventing hyperlipidemia and oxidative stress induced by HFD.

Keywords: Antioxidant, bitter gourd, high-fat diet, hyperlipidemia, hepatoprotective.

INTRODUCTION

Bitter gourd (*Momordica charantia* L, BG), also called as bitter melon and kugua, is widely planted in China, East Africa and South America. This plant has been traditionally used in folk medicine systems due to its a wide array of nutritive and pharmacological properties against many diseases such as diabetic, rheumatic, ulcer, inflammatory and cancer (Basch *et al.*, 2003; Grover and Yadav, 2004). Previous studies have shown that BG fruit contains many bioactive constituents, including cucurbitane-type triterpenoids (Lin *et al.*, 2011), flavonoids (Shan *et al.*, 2012), saponins (Keller *et al.*, 2011), phenolic compounds, etc. Among these chemical components, the major chemical constituents are triterpenoids and their glycosides, which contribute to their bitterness. A growing body of scientific literature has revealed that triterpenoids from BG fruit are associated with their antioxidant activity (Lin *et al.*, 2012) and other bioactivities (Akihisa *et al.*, 2007). Recently, phytochemical investigations revealed that cucurbitane-type triterpenoids from BG leaves (Chen *et al.*, 2009; Zhao *et al.*, 2014) had the similar or same structural characteristics with those from BG fruit (Zhang *et al.*, 2012). Because triterpenoids from BG fruit were reported to have distinct antioxidant activity, this kind of component from BG leaves was considered to have considerable probability of antioxidant activity, although there is no direct evidence until now. Furthermore, phenolic compounds were rich in BG leaves (Singh *et al.*, 2011) and the total content of phenolic acids in this leaf provided high association with free-radical scavenging activities (Kubola and Siriamornpun, 2008).

Hyperlipidemia, characterized by the increased concentrations of plasma lipid indexes including TC, TG and lipoprotein cholesterol (Akiyama *et al.*, 1996) is well known as a result of chronic HFD consumption. This dyslipidemia is generally regarded as an underlying cause of metabolic and cardiovascular disorders, such as diabetes mellitus, cardiovascular disease, fatty liver and various cancers. Because the products of plant are regarded to be cheap and have the characteristic of slight side effects, natural plants and their active constituents which are involved in lowing lipid have attracted more and more interests of the general public and physicians. The risk of hyperlipidemia would be reduced by the consumption of antioxidants from plant, such as triterpenoids and phenolic. For instance, phenolic from caesalpinia ferrea mart, panax ginseng and mulberry fruit have been used as antioxidants and could be useful in reducing lipid (Lee *et al.*, 2013; Nawwar *et al.*, 2015; Yang *et al.*, 2010). Meanwhile, the hypolipidemic effect of some plant were reported to be concerned with the antioxidant activity of triterpenoids (Rao and Gurfinkel, 2000; Santos *et al.*, 2012).

Based the information above, BG leaf is believed to be a promising source for the producing of natural antioxidant and lipid-lowering agent in functional food and pharmacy

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industry. Luckily, a few studies concerning the antioxidant effect of BG leaves or its crude extracts were available. For example, the leaf extracts of wild bitter melon exerted antioxidant activity evidenced by anitric oxide and hydroxyl radical scavenging action (Tsai *et al.*, 2014). Rahman and Iqbal reported the antioxidant activity of ethanol extracts from *Momordica charantia* evidenced by the in vitro elimination of 1, 1-diphenyl-2-picryl hydrazyl free radical (Rahman and Iqbal, 2007). However, to the best of our knowledge, the effect of hypolipidemic and antioxidant of BG leaf in the context of a HFD is poorly demonstrated. Therefore, this work was the first time to research the lowering lipid effect and the antioxidant status of BG leaf in mice fed with HDF.

MATERIALS AND METHODS

BG fruit ethanol extracts

The plant leaves collected from the local village (Xiqing district, Tianjin, China) were thoroughly washed, dried completely under the mild sun, crushed with an electric grinder and passed through a 40-mesh sieve to obtain the rough powder. 500 g sample was blended in 1500 ml of 60% (v/v) ethanol. Afterward, the mixture was extracted at 70ºC for 2 h in a water bath. The suspension was filtered through gauze, and the extraction process repeated twice at the same experimental condition. A rotary evaporator was used to condense the ethanol filtrate at 40 °C, and then the extract samples were furtherly dried to powder to obtain BGL extract (LE) using a free-dryer at -50 .The final residue was stored at -20°C until further use. LE was suspended in 1% (w/v) carboxymethyl cellulose when used in the investigation.

Animals and ethics statement

Fifty male KM mice (KM) weighing 23-26g were provided by Academy of Military Medical Science (Beijing, China). All mice received access to diet and distilled water *ad libitum* in an animal houses with conditions of temperature (25±1°C) and 12/12h Light/dark cycle. This experiment took appropriate measures to reduce pain and discomfort of the laboratory animals. The procedures involving animals followed the stand ethical guidelines for the care and use of laboratory animals at Fujian Medical University (Publication No. 85-23, revised 1985) and were approve by Tianjin Agriculture College Animal Welfare committee.

Experimental design

The healthy animals were randomly separated into five groups with 10 mice in each group after 3 days of acclimatization. table 1 shows the type of diet and concentration of LE administered to each group: NC group, basal diet; HF group, HFD (basal diet + 1% cholesterol, 0.2% cholate, 10% yolk powder and 10% lard oil); LLE，MLE，HLE group, HFD + 200, 400, 800 mg/kg LE, respectively. The experiment period was six weeks. LE was administered orally through gastric intubations using a lavage needle (size 12) to LE-treated groups. NC and HF groups were administrated through the same oral method by the same amount of distilled water. Body weight was monitored once every three days.

Serum biochemical analysis

After fasting for 12 hours, all mice were executed by cervical dislocation under ether anesthesia. The serum samples were collected by centrifuging blood (4,000 rpm/min, 4°C, and 15 minutes) and stored at -80°C until further analysis. TC, TG and HDL-C were measured spectrophotometrically according to commercial enzymatic kits provided by BioSino Bio-technology and Science Institute (Beijing, China). The content of LDL-c was calculated by the followed equation: LDL-c=TC-(HDL-c + TG/5)

Antioxidant activity assay

Liver, kidney and adipose tissues were excised and washed in phosphate buffer saline (PBS) immediately after blood collection. The weights of the organs were recorded. 9 ml of ice-cold PBS was added to 1 g of liver or kidney. The samples were then homogenized using a tissue grinder and centrifuged (3,000 rpm/min, 4°C, 10 min), the supernatant was assembled and reserved at -80°C for the further use. The MDA concentration and the antioxidant enzyme (SOD and GPX) activities were analyzed with commercial assay kits procured from Nanjing Jiancheng Biology Engineering Institute (Nanjing, China) according to the manufactures' instructions. The protein content was determined using the Coomassie blue method (Hartree, 1972), bovine serum albumin (BSA) was chosen as the standard.

Histological examination

The liver samples were collected and fixed in 10% neutral buffered formaldehyde, washed with running tap water for 30min, dehydrated using graded ethanol (50-99%), cleared using xylene, and then embedded in paraffin to prepare 5 µm coronal sections using a microtome (Model RM 2235, Germany). Sections were deparaffinized with xylene, and rehydrated using a graduated alcohol series (100-50%), then stained by hematoxylin and eosin (HE) using an auto strainer (XL, Germany). The structural abnormality of tissue was viewed under a microscope (4X-1, Japan).

STATISTICAL ANALYSIS

All data were collected and statistically analyzed by SPSS18.0. The results were expressed as the mean ± standard error. Significance of differences among group means was analyzed with one-way ANOVA. The followed multiple comparisons were analyzed by Duncan's multiple-range test. $P<0.05$ was used to indicate statistical difference.

Table 1: Type of diet and concentration of LE administrated to each group

Type of diet	Group	Treatment
Normal diet	NC	Normal diet
High-fat diet	HF	High-fat diet
	LLE	High-fat diet + 200mg/kg LE
	MLE	High-fat diet + 400mg/kg LE
	HLE	High-fat diet + 800mg/kg LE

Table 2: Effects of LE on the changes in body weight and organ index in the mice fed on a high-fat diet or normal diet

	HF	NC	LLE	MLE	HLE
IBW	25.2 ± 0.20^{NS}	25.5±0.18	25.7±0.22	25.2±0.10	25.3±0.14
FBW	45.1 ± 0.23^{a}	38.3 ± 0.56^{b}	40.1 ± 0.38^{b}	39.8 ± 1.10^{b}	38.1 ± 0.88^{b}
Weight gained (g)	19.9 ± 0.38^{a}	14.6 ± 0.51^{bc}	16.0 ± 0.99^{b}	12.9 ± 0.86^{c}	13.1 ± 0.49^{c}
%	80.0	57.3	62.3	51.2	51.8
Liver index(g/100g)	41.4 ± 1.00^{a}	36.4 ± 1.41^{b}	38.5 ± 1.38^{a}	36.9 ± 0.94^{b}	37.3 ± 0.96^{b}
Fat index (g/100g)	46.9 ± 3.03^{a}	33.9 ± 3.18^{b}	26.8 ± 1.86^{bc}	24.7 ± 2.07^{c}	22.5 ± 2.17^{c}
Kidney index (g/100g)	13.8 ± 0.44^{NS}	14.9±0.43	13.5±0.67	14.6±0.50	13.7±0.60

Data are expressed as mean ± SEM; n=10; Values with different superscripts in the same row are significantly different ($p<0.05$), NS represents not significant.

Table 3: Effects of BGEE on lipid profile and AI in the mice fed on a high-fat diet or normal diet

Group	LE Dosis (mg/kg)	TC (mmol/L)	TG (mmol/L)	LDL-c (mmol/L)	HDL-c (mmol/L)
HF	-	5.38 ± 0.19^{a}	1.87 ± 0.28^{a}	3.83 ± 0.15^{a}	1.16 ± 0.10^{a}
NC	-	3.91 ± 0.12^{b}	0.66 ± 0.05^{b}	1.51 ± 0.16^{b}	2.26 ± 0.13^{b}
LE	200	4.60 ± 0.20^{c}	0.82 ± 0.08^{b}	3.13 ± 0.16^{c}	1.31 ± 0.06^{ac}
	400	4.31 ± 0.23^{bc}	0.74 ± 0.08^{b}	2.66 ± 0.26^{c}	1.50 ± 0.12^{c}
	800	4.00 ± 0.07^{b}	0.68 ± 0.04^{b}	1.70 ± 0.07^{b}	2.16 ± 0.09^{b}

Data are expressed as mean ± SEM; n=10; Values with different superscripts in the same column are significantly different ($p<0.05$), NS represents not significant.

RESULTS

Changes in body weight

Fig. 1 shows the effects of BGE on body weight changes in the mice. During the 6-week period, the body weight in the NC group gradually increased. In contrast, the body weight of the HF group increased rapidly. Generally, the descending order of the percentages of weight gain in each group was HF>LLE>MLE>HLE> NC.

Ratio of viscera to body weight

Table 2 shows no difference in initial body weight among the five groups ($P > 0.05$). However, the final body weight of HF group was significantly enhanced when compared with NC group ($p<0.05$). The mice in LLE, MLE and HLE groups had 11.75%, 15.52%, and 15.08% lower body weight, respectively, compared with the HF mice. Organ indices for liver, abdominal fat, kidneys were measured as the ratio of viscera to body weight (g/100g). This study revealed that the fat and liver indices in the HFD administrated mice were significantly elevated ($p<0.05$). Nevertheless, the liver indices of both MLE group and HLE group were decreased ($p<0.05$) when compared with that of HF group but similar ($p>0.05$) to that of NC group. Additionally, the supplemented with LE at the three designed doses reversed the abdominal fat index increase induced by HFD in a dose-dependent manner to some extent. The kidney indexes of each group showed no significant differences ($p>0.05$).

Level of serum lipid

The hypolipidemic activity of LE was determined at doses of 200-800mg/kg BW. This study estimated the serum lipid profiles. As shown in table 3, HF animals showed an important elevation ($p<0.05$) in the plasma levels of TC, TG, LDL-c, while a significant decrease ($p<0.05$) in plasma HDL-c level compared with NC animals. Meanwhile, HFD + LE (200, 400 and 800mg/kg) administered mice showed reduced TC (14.5%, 19.9% and 25.7%), TG (56.1%, 60.4% and 63.6%), LDL-c (18.3%, 30.5% and 6%) levels and increased HDL-c(12.9%, 29.3% and 86.2%) levels compared with HF animals, all of above difference were significant ($p<0.05$).

Liver indices

As shown in fig. 2, the increased MDA level and the reduced SOD activity of HF mice was significant ($P<0.05$) when compared with NC mice. However, the oral

administration of LE at all doses significantly ($p< 0.05$) reduced the liver SOD enzymatic activity and the MDA concentration when compared with HF animals. Maximum changes of hepatic SOD and MDA of 15.4% and 12.3%, respectively, were obtained in HLE group. There were no significant ($p>0.05$) changes in hepatic GPX activities among all the experimental groups.

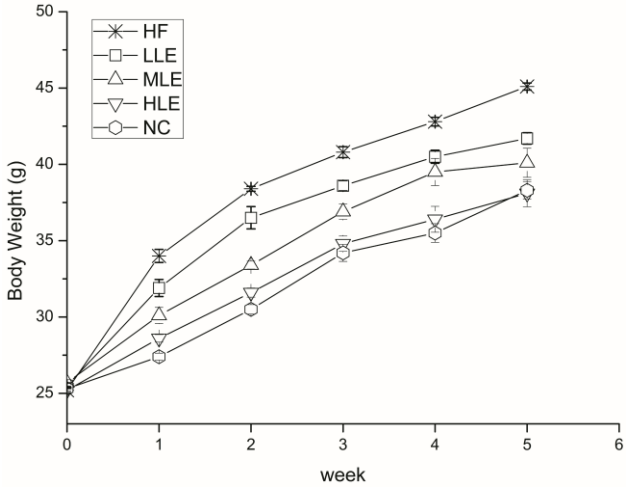

Fig. 1: Body weight of the mice fed a control diet, HFD or a diet supplemented with 200, 400, 800mg/Kg LE. Values are the mean ± SEM of 10mice per treatment group.

In fig. 3, the histopathological examination of HF group mice proved hepatic damages induced by HFD, such as inflammatory cell infiltration, hemorrhage, hepatic lobular disorganization and pyknotic nuclei. LE pretreatment at each of the doses obviously reduced these pathological damages. The intervention of LE at a dose of 800mg/kg offered the maximum protection, and the hepatic tissue structures of HLE group were nearly comparable to the normal control group. This histopathologic examination was in consistent with the results of hepatic antioxidant enzyme activities.

DISCUSSION

Many previous studies have already demonstrated that BG fruit and its extracts showed antilipidemic properties in diabetic animal models (Ahmed *et al.*, 2001; Fernandes *et al.*, 2007; Mohammady *et al.*, 2012). This study was designed to illustrate the hypolipidemic activity and the antioxidant status of LE in the context of HFD. In this study, the consumption of a HFD for 6 weeks resulted in obesity and hyperlipidemia as previous studies reported (Feng *et al.*, 2011, (Park *et al.*, 2005), evidenced by the increasing body weight and the abdominal fat index, the enhanced levels of TC, TG, LDL-c and the decreased HDL-c level in HFD feeding mice. It is well known that high blood lipid levels can result in various cardiovascular diseases (CVD) such as atherosclerosis and coronary heart disease (CHD) (Bonora *et al.*, 2003). In this work, LE dose-dependently alleviated HFD induced hyperlipidaemia. Hence, the results indicated that bitter gourd leaf could be helpful in preventing cardiovascular diseases via increasing HDL-c and lowering levels of TC, TG and LDL-c. Unfortunately, it was not established which of the components of LE contributed to this effect in current study. Saponins, widely distributed in many plants, can be classified into steroidal saponins and triterpenoid saponins based on the structure of their aglycone skeleton (Sparg *et al.*, 2004). Li et al. discovered that total saponins were isolated from the 80% ethanol extract of *Momordica charantia* L. leaves (Li *et al.*, 2015). Gamarallage et al. considered that triterpenoid saponins with a C30 backbone might behave as the major component which is responsible for ameliorating hyperlipidemia (Senanayake *et al.*, 2004). Therefore, it is easy to assume that triterpenoid saponins may be one of the main active components in lowering lipid. The contents and the structures of bioactive chemicals associated with the lipid-lowing effect in LE need to be further examined.

Lipid metabolism is governed by redistribution, storage and utilization of lipid material including lipoprotein and triacylglycerol, liver is the most crucial organ for this metabolic process (Donnelly *et al.*, 2005). High fat diets would induce hyperlipidemia and free radical production in liver tissue, thereby elevating lipid peroxides and oxidant stress (Matsuzawa-Nagata *et al.*, 2008; Ohara *et al.*, 1993). MDA is the best-known by-product of lipid per oxidation during tissue suffers from oxidative damage. In addition, MDA is more sensitive and simple when compared with other biomarker of lipid per oxidation. Hence, MDA amount is widely employed as an indication of in vivo antioxidant ability and lipid per oxidation (Ohkawa *et al.*, 1979). In this study, the HFD induced oxidative damage evidenced by the increased hepatic MDA content. However, LE distinctly reversed these changes. This finding confirmed bitter gourd leaf had antioxidant activity in vivo. As mentioned earlier, antioxidant components with effect of scavenging free radicals in vitro were detected in the leaf, such as triterpenoids and phenols. Moreover, the ethanol extract of this plant leaf has been verified to exert the potential of directly scavenging free radicals in vitro. Therefore, we considered that scavenging free radicals might be one of the mechanisms of antioxidant action of this leaf.

According to many studies, the oxidant damage is a result of defect in antioxidant system homeostasis. The natural antioxidant defense system in the body consists of antioxidant enzymes (mainly SOD and GPX) and numerous nonenzymatic antioxidant compounds. SOD converts the O^{2-} radicals into hydrogen peroxide (H_2O_2), and the generated H_2O_2 can be catalyzed to H_2O by GPX enzyme (Rocha *et al.*, 2009). In present study, EE pretreatment, especially at doses of 800mg/kg, markedly

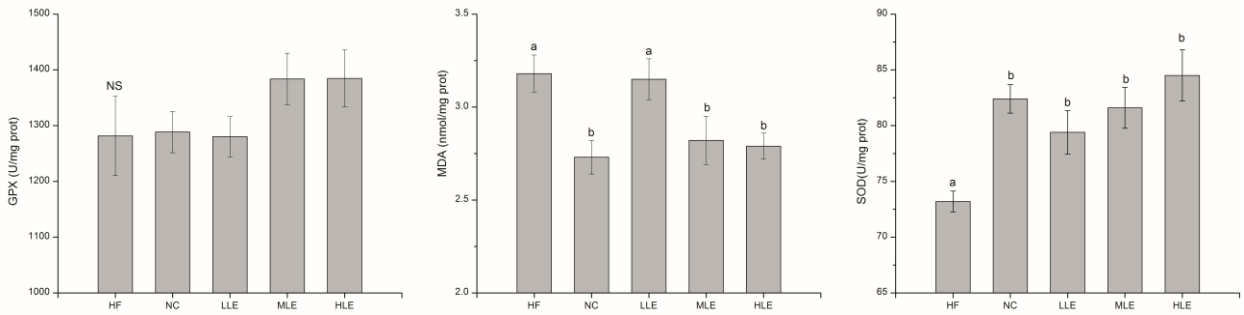

Fig. 2: Effect of ethanol extract of bitter gourd leaves (LE) on the MDA level and the activities of SOD and GPX in the liver of HFD mice. Results are presented as the means ± SE (n = 10 animals in each group). Group NC: normal control; Group HF: HFD administration; Group LLE: HFD + 200 mg/kg LE; Group MLE: HFD+400 mg/kg LE; and Group HLE: 800 mg/kg LE. Notes: NS = not significant. a Significance at $p<0.05$ compared with Group NC. b Significance at $p<0.05$ compared with the Group HF.

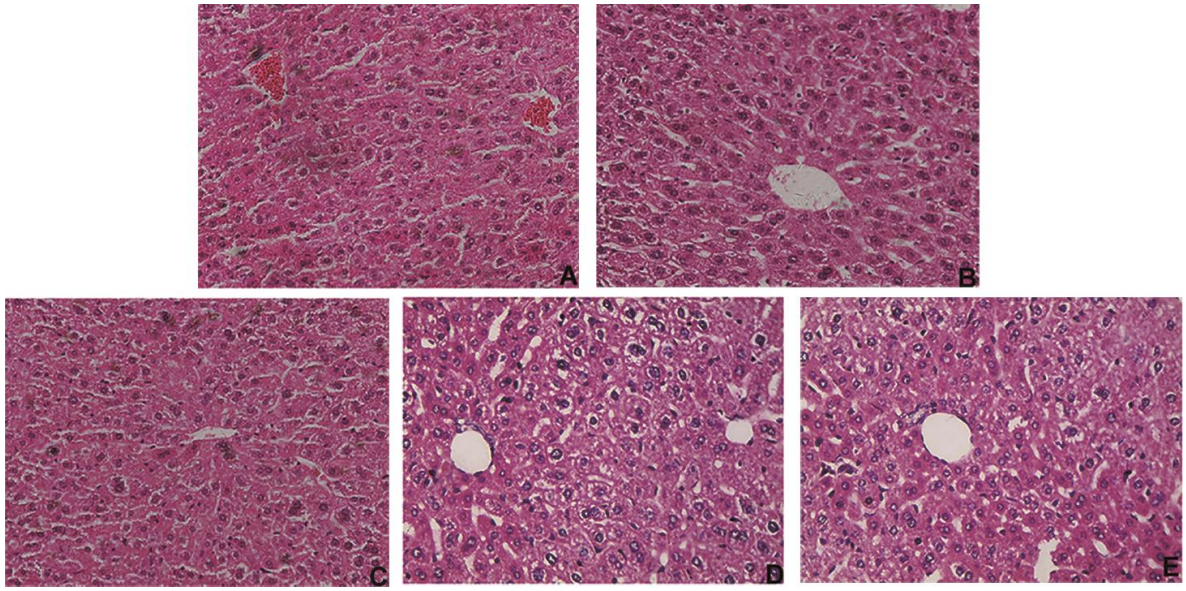

Fig. 3: The Protective effects of ethanol extract of bitter gourd leaves (LE) against hyperlipidemia in mice treated with a high fat diet (original magnification of ×400). (A) Group HF (HFD-treated group) showing a severe damage of hepatic architecture g; (B) Group NC (normal diet treated group) showing normal histological structure. (C, D, E) Group LLE, MLE and HLE (HFD + 200, 400, 800mg/kg LE respectively) showing ameliorative cellular architecture close to normal;

enhanced the impaired hepatic SOD activities, suggesting that LE, acting as an antioxidant agent, exerts hepatoprotective effect by ameliorating oxidative stress in the damaged liver tissue. This liver-protection property of EE was further confirmed by our histopathological examination. Hence, BG leaf could suppress the development of oxidative damage by partly enhancing antioxidant enzymes activities as an antioxidant. This may be another antioxidant mechanism for bitter gourd leaf. But, it is interesting to note that LE administrating have no effect on GPX in liver, more information about the effect of bitter gourd leaf on antioxidant system *in vivo* need to be collected in the next study.

CONCLUSION

In conclusion, our study was the first to examine the effectiveness of bitter gourd leaf on lowing-lipid in HFD feeding mice. The mechanism may be related to potentiating the antioxidant enzyme activities and suppressing the development of oxidative stress. Although this study did not analyze the active composition of LE, this report did propose to investigate the ethanol extract of BG leaf for the beneficial effects against hyperlipidaemia, oxidative stress, obesity, etc. Further studies are needed to focus on identifying the bioactive constituent(s), and the intimate mechanism(s) involved in lipid-metabolism and antioxidant activity that could explain the effect of BG leaf.

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