ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 11, Special issue 3, 2018

Online - 2455-3891
Print - 0974-2441

Research Article

ANTI-HYPERLIPIDEMIC ACTIVITY OF METHANOLIC EXTRACT OF *BOESENBERGIA PANDURATA* (FINGER ROOT) IN EXPERIMENTAL INDUCED HYPERCHOLESTROLEMIC SPRAGUE DAWLEY RATS

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Received: 22 December 2018, Revised and Accepted: 19 April 2018

ABSTRACT

Objective: Hyperlipidemia is one of the risk factors that contribute to the prevalence of coronary heart diseases and antihyperlipidemic agents, such as statin, was used to treat hyperlipidemia as a current therapy. *Boesenbergia pandurata* has not been exploited for antihyperlipidemic effect. Hence, this study aims to screen for the antihyperlipidemic activity of methanolic extracts of *B. pandurata* rhizomes (BPR extracts) in hypercholesterolemia-induced Sprague-Dawley rats.

Methods: BPR extracts were prepared using the maceration method with 1500 ml of 80% methanol at room temperature for about 7 days. A toxicity study was carried out based on OECD guidelines. Hypercholesterolemia was induced by 6% lard oil, 2% of cheese, and egg yolks. Two different doses of BPR extracts, 200 and 400 mg/kg, were used to screen for antihyperlipidemic effect. Histopathological study was carried out in the liver. The results were evaluated for the statistically significant difference by using the one-way ANOVA followed by *post hoc* Dunnett test.

Results: No mortality was witnessed even till 2 g/kg. Only 400 mg/kg of *BPR* extracts statistically reduced in total cholesterol ($p<0.05$), low-density lipoprotein-cholesterol ($p<0.05$) and an increase in high-density lipoprotein-cholesterol ($p<0.05$) when compared to the positive control. BPR extracts (400 mg/kg) showed less enlargement of lipid droplets as compared to positive control.

Conclusion: *BPR* extracts can be a promising medicinal plant for treating hyperlipidemia in underdeveloped countries.

Keywords: Anti-hyperlipidemia, Hypercholesterolemia, Statins, Finger root (*Boesenbergia pandurata*).

 DOI: http://dx.doi.org/10.22159/ajpcr.2018.v11s3.29962

INTRODUCTON

Hyperlipidemia is known as a condition of increased level of serum total cholesterol (TC), low-density lipoprotein (LDL), very LDL (VLDL), and reduced high-density lipoprotein (HDL). Hyperlipidemia has been sorted as one of the high-risk factors that lead to the prevalence and severity of coronary heart diseases [1]. The desired level of TC is <200 mg/dL, LDL is <130 mg/dL, triglycerides is <120 mg/dL, and HDL for men is >40 mg/dL while for women is >50 mg/dL. Hyperlipidemia can be categorized into two groups which are the primary and the secondary. The primary hyperlipidemia can be further divided into five types. The second type is better known as the secondary causes of hyperlipoproteinemia [2].

In modern globalization era, there is almost treatment for all diseases including treatments for hyperlipidemia. Many antihyperlipidemia therapies such as statin and fibrates that aid in correcting the altered blood lipid profile by hindering the formation of cholesterol and by improving the clearance of triglycerides rich lipoproteins [3]. Many drugs used for the treatment are found to be associated with side effects when used. These may lead to hyperuricemia, diarrhea, nausea, myositis, gastric irritation, flushing, dry skin, and also abnormal liver function [4]. Natural sources are believed to have lesser side effects than the available therapy in the market. Therefore, it is important to find other essential materials from natural sources which are low in toxic, low in cost, and provides better safety and efficacy on a long-term usage. It has been for centuries that natural products from a plant source are used to cure various ailments [5].

Many researches have been done on *Boesenbergia pandurata* which is profoundly found all over Indonesia, Thailand, and Malaysia. The plant extract has proven antibacterial, antifungal, anti-inflammatory, analgesic, antipyretic, antispasmodic, antitumor, and insecticidal activities [6]. Till date, it lacks antihyperlipidemic activity. Therefore, the study has been conducted to screen the antihyperlipidemic properties of *B. pandurata* rhizomes (BPR) extracts in rats.

MATERIALS AND METHODS

Collection of plant materials

The fresh rhizomes of *B. pandurata* were collected from local area in Johor Bahru, Malaysia, and got authenticated from FRIM (Forest Research Institute Malaysia).

Maintenance of animals and approval of protocol

Healthy Sprague-Dawley (SD) rats weighing between 100 and 150 g were obtained from the local vendor and placed in the polypropylene cages maintained at 25±2°C in Management and Science University animal house. Throughout the study, they are maintained on standard pallet and water *ad libitum*. Institutional Animal Ethical Clearance was obtained before the study.

Preparation of extract

The rhizomes of the *B. pandurata* were sliced, shade-dried, and powdered using the mechanical grinder. 500 g of the powdered rhizomes was macerated with 1500 ml of 80% methanol at room temperature for 7 days, and the final product was filtered using a muslin cloth and filter paper. The extract was concentrated to dryness

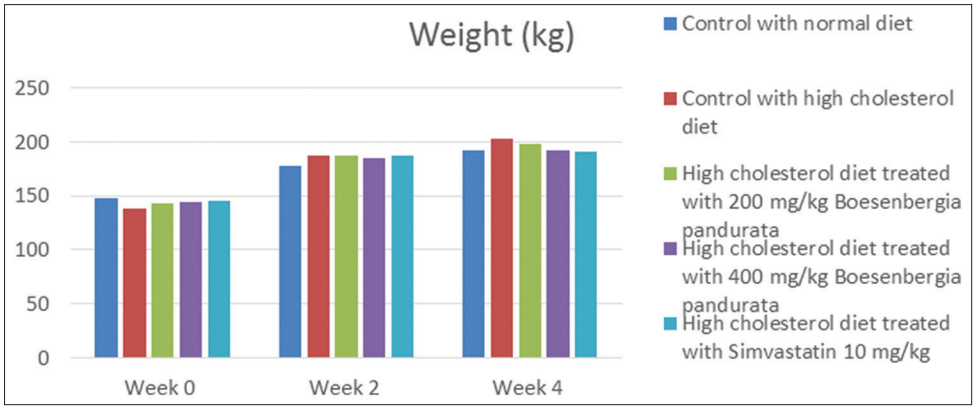


Fig. 1: Antihyperlipidemia effect of *Boesenbergia pandurata* rhizomes extract 200 mg/kg and 400 mg/kg in hyperlipidemia-induced rats. The values of weight are expressed as mean±SEM of six rats

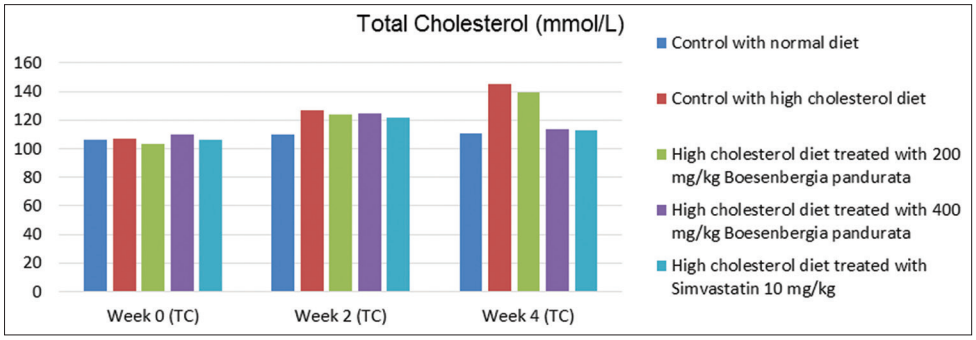


Fig. 2: Antihyperlipidemic effect of *Boesenbergia pandurata* rhizomes extract 200 mg/kg and 400 mg/kg in hyperlipidemia-induced rats. The values of total cholesterol are expressed as mean±SEM.

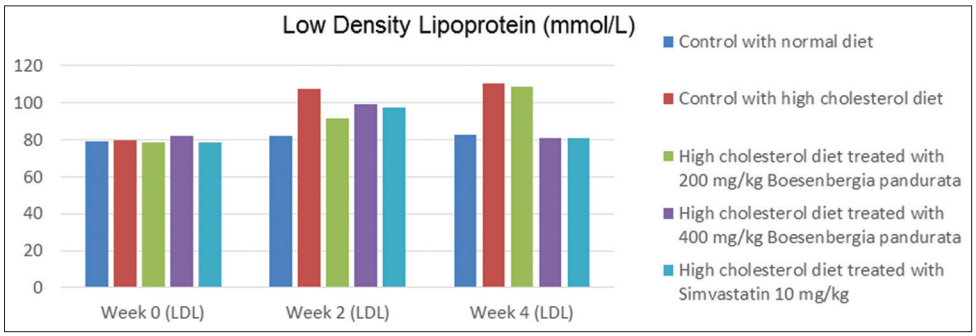


Fig. 3: Antihyperlipidemic effect of *Boesenbergia pandurata* rhizomes extract 200 mg/kg and 400 mg/kg in hyperlipidemia-induced rats. The values of low-density lipoprotein are expressed as mean±SEM

under reduced pressure and controlled temperature (40–50°C) in rotatory evaporator to remove the methanol. The brownish extract was scrapped out and weighed to quantify the yield value and also other organoleptic properties. The yielded BPR extract was kept at 4°C (cold room) until further use [7].

Toxicity study

A single dose of 500 mg/kg, 1 g/kg, and 2 g/kg of *BPR* extract was given by intragastric intubation by gavage needle to SD rats (n=3). The animals were observed for mortality and toxicity signs for 14 days. The animals were observed individually at least once during the first 30 min, periodically during the first 4 h, and daily thereafter, for a total of 14 days.

Experimental design (high-cholesterol diet-induced hyperlipidemia in rats)

The animals were divided into five groups (n=6) as follows. Group 1: Normal control rats fed with normal diet for 4 weeks; Group 2: Animals fed with high-cholesterol diet for 4 weeks; Group 3: Animals fed with high-cholesterol diet for 4 weeks. During the last 2 weeks, 200 mg/kg of *BPR* extract was administered daily by intragastric intubation; Group 4: Animals fed with high-cholesterol diet for 4 weeks. During the last 2 weeks, 400 mg/kg of *BPR* extract was administered daily by intragastric intubation; and Group 5: Animals fed with high-cholesterol diet for 4 weeks. During the last 2 weeks, the reference standard drug Simvastatin (10 mg/kg) was administered daily by intragastric intubation.

Preparation of high-cholesterol diet

Eggs and high-cholesterol cheese were procured from local market at Klang Valley, Malaysia. The lard oil was prepared using rendering method where mutton fats were bought from Masai, Johor Market, and chopped to fine cubes. These fat cubes were placed in a large stockpot and heated slowly, delicately over the medium heat. The fat was stirred at regular interval for 30min. The high-cholesterol diet was prepared by mixing 60ml of melted lard oil, with 2 egg yolks, and 20ml of melted cheese. These ingredients were mixed at mild heat water bath to prevent solidification

of oil and cheese at room temperature. This high-cholesterol diet was fed to the SD rats for 4 weeks according to their body weight by using gavage needle. Each rat was weighed and received 20ml/kg of this semisolid high-cholesterol diet twice daily (morning and evening) [8].

Biochemical analysis

The enzymatic kit was used to assay the TC, LDL-cholesterol, and HDL-cholesterol. The blood biochemical analysis was done by using ACON MISSION Cholesterol meter [9].

Histopathological assessment

The liver sections of the SD rats were fixed in 10% formaldehyde, dehydrated in gradual ethanol (50–100%), cleared in xylene, and embedded in paraffin. Sections (4–5 µm thick) were prepared and stained with hematoxylin and eosin (HE) dye and observed under a microscope.

Statistical analysis

The results were evaluated for the statistically significant difference using the one-way ANOVA followed by *post hoc* Dunnett test using SPSS software version 24. A statistically significant difference was accepted at the level of $p<0.05$.

RESULTS

Toxicity study

There were no toxic symptoms or mortality was observed or recorded in all the animals that have been given with 500 mg/kg, 1 g/kg, and 2 g/kg of the BPR extract (Figs. 1-5).

Histopathological result

Histopathological examinations were performed on the liver to assess whether the tissue have been damaged or not. The results indicated that rats treated with high cholesterol diet and also the rats treated with high cholesterol diet treated with 200 mg/kg BPR extract showed swelling in liver cells and also steatosis. However the animals treated high cholesterol diet treated with 400 mg/kg BPR extract showed reduction in swelling and steatosis. The results are shown in the Table 6.

DISCUSSION

It is well known that nutrition plays an essential role in causing hyperlipidemia and atherosclerosis. Fat diet was used in the study

Table 1: Antihyperlipidemic effect of BPR extracts 200 mg/kg and 400 mg/kg in hyperlipidemia-induced rats

Groups	Treatment	Weight (kg)		
		Week 0	Week 2	Week 4
I	Normal standard diet	147.75±1.62	177.68±1.67	192.56±1.49
II	High-cholesterol diet	138.26±1.87	187.58±1.20	202.60±1.86
III	High-cholesterol diet treated with 200 mg/kg BPR extract	142.49±3.18	187.83±1.05	198.13±1.91
IV	High-cholesterol diet treated with 400 mg/kg BPR extract	143.63±1.54	185.41±1.39	191.49±1.60
V	High-cholesterol diet treated with simvastatin (10 mg/kg)	145.69±1.50	187.45±2.61	190.90±1.51

The values of weight are expressed as mean±SEM. BPR: *Boesenbergia pandurata* rhizomes

Table 2: Antihyperlipidemic effect of BPR extracts 200 mg/kg and 400 mg/kg in hyperlipidemia-induced rats

Groups	Treatment	TC mmol/L		
		Week 0	Week 2	Week 4
I	Normal standard diet	106.02±2.24	109.59±2.47	110.54±2.11
II	High-cholesterol diet	106.59±1.40	126.44±1.53	145.42±2.37
III	High-cholesterol diet treated with 200 mg/kg BPR extract	103.46±1.47	123.86±2.18	139.34±3.52[b]
IV	High-cholesterol diet treated with 400 mg/kg BPR extract	110.13±1.58	124.46±2.48	113.42±2.11[a]
V	High-cholesterol diet treated with simvastatin (10 mg/kg)	106.33±1.73	121.83±3.89	112.67±4.57[a]

The values of TC are expressed as mean±SEM. [a]$p<0.05$ compared with positive control. TC: Total cholesterol, BPR: *Boesenbergia pandurata* rhizomes

Table 3: Antihyperlipidemic effect of BPR extract 200 mg/kg and 400 mg/kg in hyperlipidemia-induced rats

Groups	Treatment	LDL mmol/L		
		Week 0	Week 2	Week 4
I	Normal standard diet	79.37±2.16	82.33±1.35	82.74±0.93
II	High-cholesterol diet	79.53±2.29	107.75±3.19	110.56±4.17
III	High-cholesterol diet treated with 200 mg/kg BPR extract	78.24±2.26	91.63±3.31	108.44±1.65[b]
IV	High-cholesterol diet treated with 400 mg/kg BPR extract	81.80±2.26	99.36±5.30	81.10±1.23[a]
V	High-cholesterol diet treated with Simvastatin (10 mg/kg)	78.45±2.26	97.33±2.31	81.08±2.52[a]

The values of LDL are expressed as mean±SEM; [a]$p<0.05$; [b]$p>0.05$ compared with positive control. LDL: Low-density lipoprotein, BPR: *Boesenbergia pandurata* rhizomes

Table 4: Antihyperlipidemia effect of BPR extract a 200 mg/kg and 400 mg/kg in hyperlipidemia-induced rats

Groups	Treatment	LDL mmol/L		
		Week 0	Week 2	Week 4
I	Normal standard diet	12.46±0.89	12.94±0.90	12.60±0.92
II	High-cholesterol diet	12.60±1.23	12.04±1.08	11.49±1.07
III	High-cholesterol diet treated with 200 mg/kg BPR extract	12.67±0.97	12.95±1.14	11.28±1.32[b]
IV	High-cholesterol diet treated with 400 mg/kg BPR extract	12.08±1.26	12.43±1.20	15.84±1.09[a]
V	High-cholesterol diet treated with simvastatin (10 mg/kg)	12.25±1.03	12.19±0.92	16.97±0.98[a]

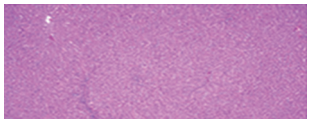
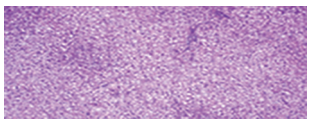
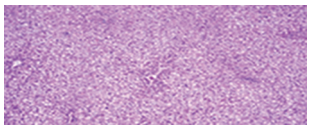
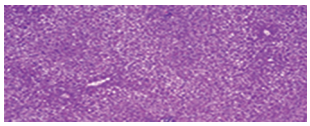
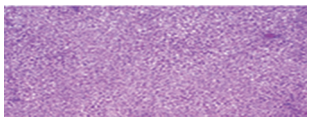
The values of HDL are expressed as mean±SEM; [a]$p<0.05$; [b]$p>0.05$ compared with positive control. BPR: *Boesenbergia pandurata* rhizomes, LDL: Low-density lipoprotein

Table 5: Antihyperlipidemia effect of BPR extracts 200 mg/kg and 400 mg/kg in hyperlipidemia induced rats

Groups	Treatment	Percentage increase (+) or decrease (−) of lipid values at week 4		
		TC	LDL	HDL
I	Normal standard diet	110.54±2.11	82.74±0.93	12.60±0.92
II	High-cholesterol diet	145.42±2.37	110.56±4.17	11.49±1.07
III	High-cholesterol diet treated with 200 mg/kg BPR extract	139.34±3.52 (−4.18%)	108.44±1.65 (−1.92%)	13.28±1.32 (+15.58%)
IV	High-cholesterol diet treated with 400 mg/kg BPR extract	113.42±2.11 (−22.01%)	87.10±1.23 (−21.22%)	15.84±1.09 (+37.86%)
V	High-cholesterol diet treated with Simvastatin (10 mg/kg)	112.67±4.57 (−22.52%)	81.08±2.52 (−26.66%)	16.97±0.98 (+47.69%)

The values of percentage increase and decrease in the lipid values at the last week with reference to the positive control are shown below. BPR: *Boesenbergia pandurata* rhizomes, LDL: Low-density lipoprotein, HDL: High-density lipoprotein, TC: Total cholesterol

Table 6: Histopathological results of the liver of rats with normal diet, high-cholesterol diet and high-cholesterol diet with BPR extracts 200 mg/kg and 400 mg/kg in hyperlipidemia-induced rats

Groups	Histopathological results
Normal standard diet  Liver cell is normal	High-cholesterol diet  Liver cells swell with steatosis
High-cholesterol diet treated with 200 mg/kg *BPR* extract  Liver cells swell with steatosis	 High-cholesterol diet treated with 400 mg/kg *BPR* extract Reduction in the swelling of liver cells and reduction in steatosis
High-cholesterol diet treated with Simvastatin (10 mg/kg)  Reduction in the swelling of liver cells, reduction in steatosis, and restoration in the liver tissue morphology	

BPR: *Boesenbergia pandurata* rhizomes

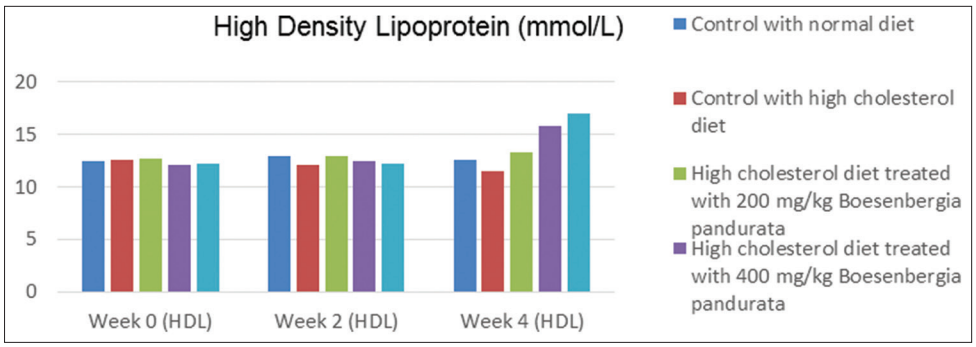


Fig. 4: Antihyperlipidemia effect of *Boesenbergia pandurata* rhizomes extract 200 mg/kg and 400 mg/kg in hyperlipidemia-induced rats. The values of high-density lipoprotein are expressed as mean±SEM

which contains some of the common ingredients in our daily. High-cholesterol diet will encourage the rate of cholesterol formation. Moreover, this can also lead to the elevation of serum LDL [8]. It is well known that an increased level of serum LDL-cholesterol will result in an increased threat for the development of atherosclerosis. In the opposite, an increase HDL-cholesterol levels will have a defensive role in the coronary artery disease [5]. Simvastatin is a potent HMG-CoA reductase inhibitor that has the ability to reduce plasma cholesterol levels in hypercholesterolemia patients. Rats treated with Simvastatin showed a marked reduction in all serum lipoproteins and increase in HDL level as compared with high-cholesterol diet group.

No toxic symptoms or mortality was observed or recorded in all the rats that have been given 500 mg/kg, 1 g/kg, and 2 g/kg of *BPR* extract. The

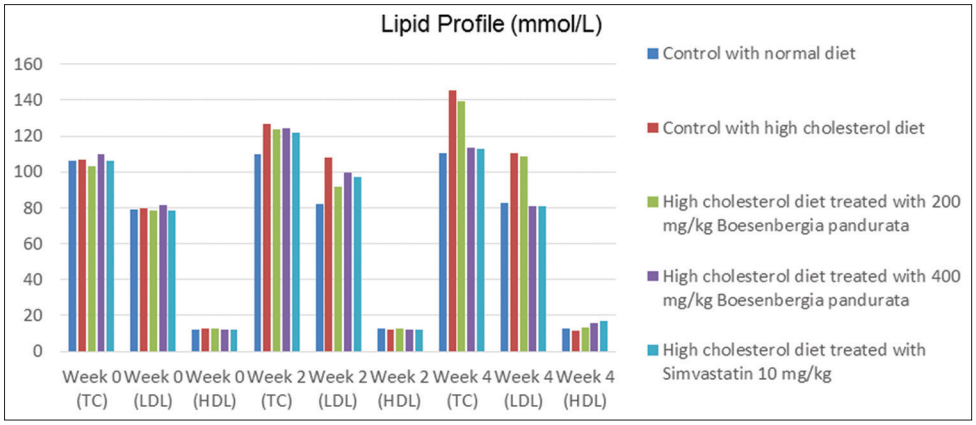


Fig. 5: Antihyperlipidemia effect of *Boesenbergia pandurata* rhizomes extracts 200 mg/kg and 400 mg/kg in hyperlipidemia-induced rats. The values of percentage increase and decrease in the lipid values at the last week with reference to the positive control are shown below

antihyperlipidemia study was conducted by measuring the body weight, and lipid parameters such as TC, LDL, and HDL by using the lipid kits before the study (week 0), during the study (week 2), and also after the study (week 4). The percentage of lipid-lowering was also calculated on the 4th week which is at the end of the study. The results obtained from the study were shown in Tables 1-6. Based on the observation, it shows there is a significant increase in the body weight of animals after inducing hypercholesterolemia in the rats. From the study, it showed that the administration of *BPR* extract at the dose of 200 mg/kg does not significantly reduce the high-cholesterol levels of the rats, while the administration at the dose of 400 mg/kg does significantly reduce the high-cholesterol levels of the rats when compared to the positive control group.

From the results obtained, it showed that the animal on high-cholesterol diet had increased TC, increased LDL-C, and decreased HDL-C when compared to the rats on the normal diet. When high-cholesterol diet was coadministered with 400mg/kg of BP extracts, the increased levels of TC and LDL-C had shown significant reduction while decreased levels of HDL-C has shown significant elevation. This indicates that the ability of 400mg/kg *BPR* extract in preventing the incensement seen in lipid profile under experimentally induced hypercholesterolemia. From previous studies, it showed that flavonoids have the ability to reduce LDL-C and increase HDL-C in hypercholesterolemia-induced rats [9]. Hence, in this study, flavonoids presence in *BPR* extract might be the reason for reducing TC, LDL-C, and increasing HDL-C in 400mg/kg treated rats.

CONCLUSION

In conclusion, the findings of the study revealed that the *BPR* extract at a dose of 200 mg/kg does not possess antihyperlipidemia properties, while at a dose of 400 mg/kg, it does possess antihyperlipidemia properties when compared with the positive control group. The results of the data have supported the presence of antihyperlipidemia effect of *BPR* extract at a dose of 400 mg/kg.

ACKNOWLEDGMENT

The author would like to thank Professor Tan Sri Dato' Wira, Dr Mohd Shukri Ab Yajid, President, Management & Science University, for providing financial support to carry out the research project and publish.

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