Research Article

Malays. j. med. biol. res.

Efficacy of *Myrmecodia Platytyrae (MyP)* Water Extract in Reducing Cholesterol Level in Hypercholesterolemia Induced Sprague Dawley Rat

M. K. Nik Hasan[1*], I. Abdul Wahab[2], H. H. Mizaton[3], M. A. Rasadah[4]

[1,2,3]Faculty of Pharmacy, Universiti Teknologi MARA (UITM), Bandar Puncak Alam, Selangor, 42300, **MALAYSIA**
[4]Natural Product Division, Forest Research Institute Malaysia, 52109 Kepong, Darul Ehsan, Selangor, **MALAYSIA**

[*]Email for Correspondence: mohdkamal@frim.gov.my

ABSTRACT

Myrmecodia plant or ant-nest plant is from Rubiaceae family. Rubiaceae are mainly tropical woody plants, consist mostly of trees and shrubs and can be found in temperate regions. *Myrmecodia platytyrea* (*MyP*) are believed to have medicinal value. This study was designed in order to investigate the effect of *MyP* extract as anti hypercholesterolemic agent. The results showed that treatment of *MyP* can significantly reduce ($p<0.05$) low density lipoprotein (LDL) compared to negative control group. The extract was significantly increase ($p<0.05$) high density lipoprotein (HDL) concentration compared to negative control group. Besides that, *MyP* increased fecal cholesterol and fecal bile compared to normal control group. It was also found that lipid profile was significantly decreased ($p<0.05$) in *MyP* treatment group. All biochemistry data showed that *MyP* water extract was not toxic at all.

Keywords: Myrmecodia platytyrea, anti hypercholesterol, low density lipoprotein, high density lipoprotein

Manuscript Received: 15 April 2020 - Revised: 20 June 2020 - Accepted: 10 July 2020

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INTRODUCTION

Hypercholesterolemia is a vital factor for the development of cardiovascular diseases such as atherosclerosis (Saba *et al*., 2016). Hypercholesterolemia is due to high levels of low-density lipoprotein (LDL) (de Isla *et al*., 2016) and frequently associated with low values of high-density lipoprotein (HDL) (Tolani *et al*., 2013). Oxidised low-density lipoprotein (ox-LDL) and endothelial dysfunction have been established to play a key role in atherosclerosis (Narasimhulu *et al*., 2016). In herbal plants, phenolic compounds have been shown to have beneficial antioxidant constituents for human health (Bordoloi *et al*., 2016; Šibul *et al*., 2016). Many polyphenolics exert more powerful antioxidant effect (Paun *et al*., 2016) and inhibit lipid peroxidation (Shodehinde *et al*., 2013). They can also directly scavenge reactive oxygen species (ROS), such as superoxide, peroxynitrite and hydroxyl radicals (Morita *et al*., 2016). Previous studies showed that compounds from the plant can directly decrease cholesterol production in liver cells (Cao *et al*., 2013; Hamidpour *et al*., 2015). Anticholesterol and antioxidant effects are two important characteristics of compounds that are needed in order to successfully reduce cholesterol related diseases (Xie *et al*., 2011; Hopkins *et al*., 2013; Ogunremi *et al*., 2015).

One of traditional medicine plant that was used by people of Papua New Guinea, Vietnam and Indonesia which is not well identified is Sarang semut or *Myrmecodia* (Soeksmanto *et al*., 2010). From this species, there are only *Myrmecodia pendens* (*M. pendens*) and *Myrmecodia tuberose* (*M. tuberose*) are believed to have medicinal value (Soeksmanto *et al*.,

2010). However, recently, *Myrmecodia platytyrae* (*MyP*) which is one of *Myrmecodia* genus was proven to have anti-cancer properties and not toxic to normal cell (Mizaton *et al*., 2010). Engida *et al*., (2013) reported that *Myrmecodia* genus contain high in flavonoid and act as good antioxidant. Recently, a study was done by Roslizawaty *et al*., (2015) showed that treatment of 100 mg and 200 mg ethanol *Myrmecodia sp.* extract can reduce cholesterol level in the rat that was induced with hypercholesterolemia. This animal laboratory study was done in order to determine the effect of *MyP* water extract to contribute anticholesterol effects on hypercholesterolemic induced rat.

METHOD

Preparation of 10% *MyP* Water Extract

Ten percent (10%) of *MyP* water extract was prepared by adding 100g *MyP* powder to 900 ml distilled water and boiled at 100ºC for 15 hours. The solution was filtered and the supernatant was concentrated by using rotavap at 50ºC. The processed 10% water extract of *MyP* was stored at -80ºC until use.

Induction of Hypercholesterolemia in Rats and its Treatment

Experiments were approved by the Ethical Committee of the Institution Animal Care and Use Committee – Forest Research Institute Malaysia (IACUC-FRIM). Thirty-six male Sprague–Dawley rats (7 weeks old) were housed separately (3 animals/cage). The rat was kept in a temperature-controlled (25 ± 2°C) room with a regular 12 h light: 12 h dark cycle. After acclimatisation period for a week in this laboratory environment, all the rats were then randomly assigned to six experimental groups (n = 6 for each group) like below:

A) Normal Control group (Normal) was fed with standard normal rat chow with protein (~14%), fat (~10%) and carbohydrate (~76%).
B) Negative Control group (NC) was fed with high cholesterol diet (HCD). The diet was formulated by using standard rat chow plus 60% pure cholesterol, 1% cholic acid and 5% peanut oil.
C) Positive Control group (PC) was Simvastatin treatment group. The PC group was fed with HCD and treated with 5mg/kg Simvastatin.
D) The 100 mg *MyP* water extract treatment group (*MyP* 100) was fed with HCD and treated with *MyP* (100 mg/kg).
E) The 200 mg *MyP* water extract treatment group (*MyP* 200) was fed with HCD and treated with *MyP* (200 mg/kg).
F) The 400 mg *MyP* water extract treatment group (*MyP* 400) was fed with HCD and treated with *MyP* (400 mg/kg).

All rats were given free access to water and their corresponding diet throughout the experimental period. Food intake and body weight were monitored every 1 week.

Faecal Cholesterol Analyses

At week 3, the faeces were collected during the last three days using metabolic cages and used to determine the faecal cholesterol. The faeces was dried, milled, and stored at −80°C until used. For extraction process, 15 ml of 3: 1 alcohol-ether mixture was added in a 25 ml volumetric flask containing 0.5 mg of faeces. The mixture was allowed to stand for 30 min at room temperature with occasional swirling and mixed by slow inversion at least ten times. The mixture was filtered through a Whatman No. 41 filter paper. Two millilitres aliquot was used for total cholesterol determination by using the kit.

Faecal Bile Acid Analyses

The faeces from individually housed rat were collected, weighed, and dried. Then 0.5 g of dried faeces was minced and extracted with 10 ml of 75% EtOH at about 50°C for 2 h. The extract was centrifuged, and a 1ml sample of supernatant was diluted to 4 ml with a 25% PBS solution for faecal bile assay. A number of faecal bile acids were determined enzymatically by using the bile acids kit (Sigma).

Termination and Blood Collection

At the end of the week 4 experimental period, animals subjected to 12 hours fasting period to allow relevant estimation of biochemical parameters. The animals were sacrificed by using diethyl ether. Blood sampling via cardiac puncture at an approximate volume of 8 ml from each rat was drawn into EDTA tube for plasma preparation. The tubes were centrifuged at 3000 rpm for 10 minutes at 25ºC. Plasma obtained from the centrifugation was aliquoted into Eppendorf tubes. All samples were stored at −80 °C until analysed.

Biochemistry test

The plasma was analysed its biochemistry profile by Roche commercial kit. The kit was used to measure the level of ALT, AST, ALP, Creatinine Kinase (CK) and Urea in the plasma using biochemistry analyzer at the Bioactivity Laboratory, Natural Products Division, FRIM.

Plasma Lipid Profile

Analysis of lipid profile includes measuring TC, TG, LDL and HDL levels in the plasma by using Roche commercial kit.

Statistical analysis

All data are presented as means ± standard deviation (SD), and n denotes the number of replications for each data point. Differences between groups were analysed using one-way analysis of variance (SPSS, Version 20). Post hoc tests were performed for inter-group comparisons and p value of <0.05 was considered significant.

RESULTS

Food Intake

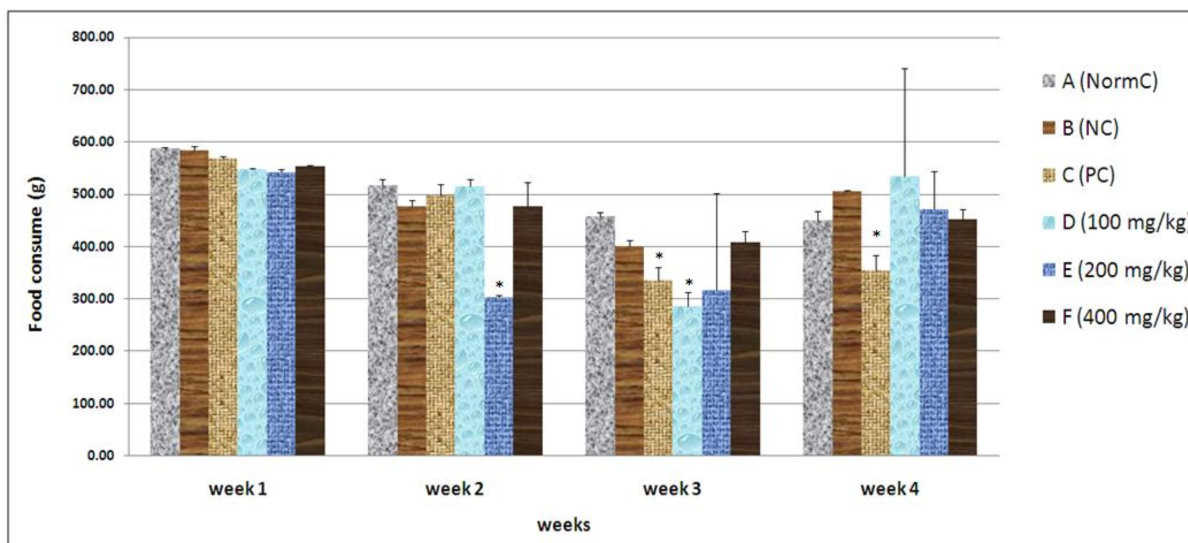

Figure 1. Food intake in rats treated with *MyP* water extract for 4 weeks. Results are expressed as mean ± S.D. Different superscripted letters on top of the chart indicate statistical differences ($p < 0.05$). Chart with an asterisk (*) symbol indicating significantly different from the normal control group ($p < 0.05$) for each week. n = 6.

Water Intake

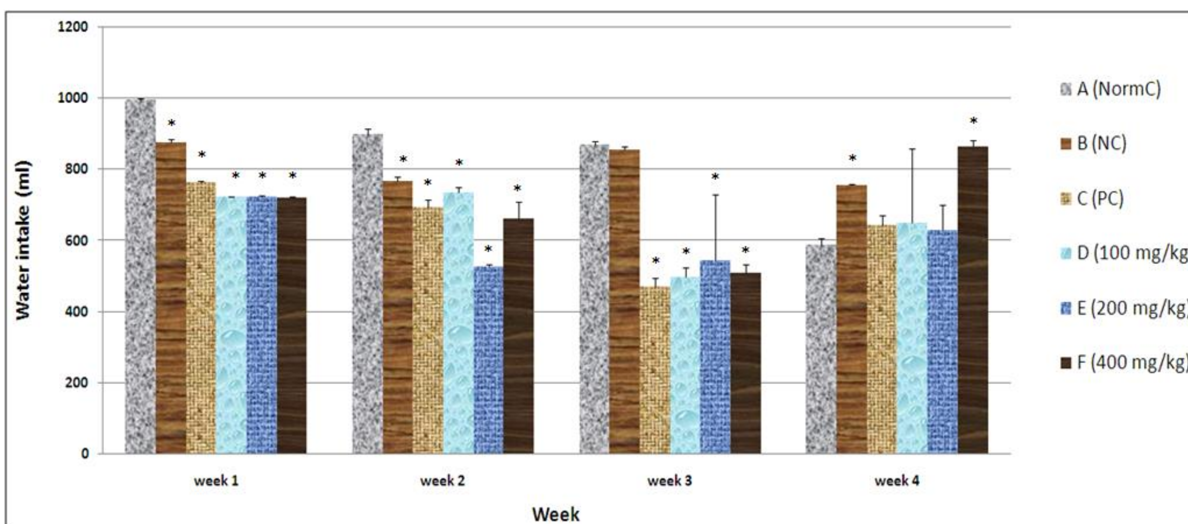

Figure 2. Water intake in rats treated with *MyP* water extract for 4 weeks. Results are expressed as mean ± S.D. Different superscripted letters on top of the chart indicate statistical differences ($p < 0.05$). Chart with an asterisk (*) symbol indicating significantly different from the normal control group ($p < 0.05$) for each week. n = 6.

Bodyweight

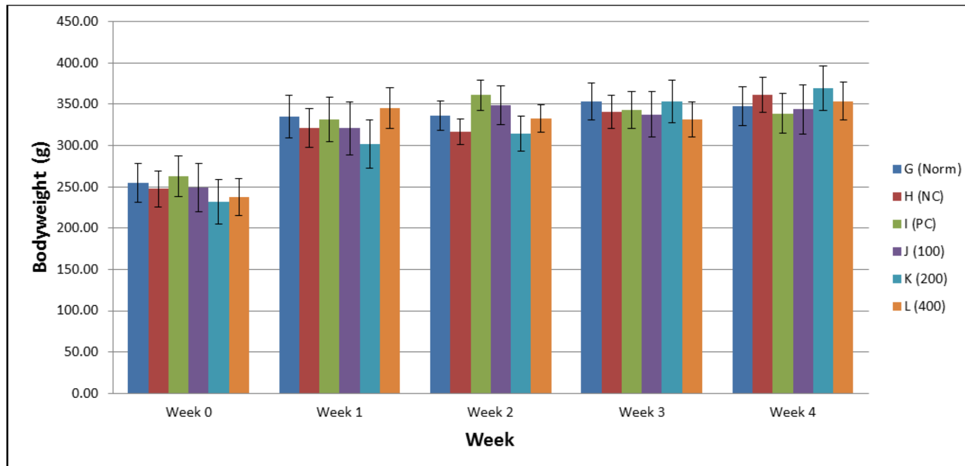

Figure 3. Body weight of rats treated with *MyP* water extract for 4 weeks. Results are expressed as mean ± S.D. Different superscripted letters on top of the chart indicate statistical differences ($p < 0.05$). Chart with an asterisk (*) symbol indicating significantly different from the control group ($p < 0.05$) for each week. n = 6.

Plasma Lipid Profile

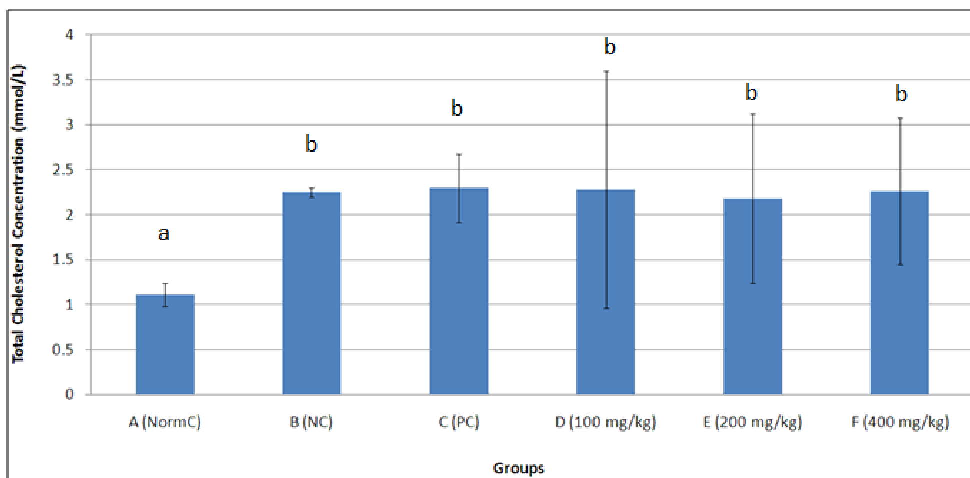

Figure 4. Total cholesterol concentration in rat's plasma treated with *MyP* water extract for 4 weeks. Results are expressed as mean ± S.D. Different superscripted letters on top of the chart indicate statistical differences ($p < 0.05$). n = 6.

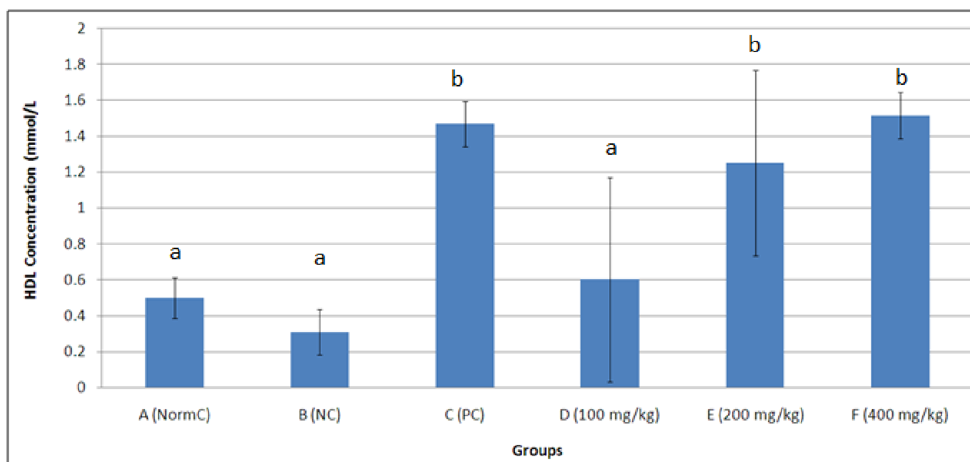

Figure 5. HDL concentration in rat's plasma treated with *MyP* water extract for 4 weeks. Results are expressed as mean ± S.D. Different superscripted letters on top of the chart indicate statistical differences ($p < 0.05$). n = 6.

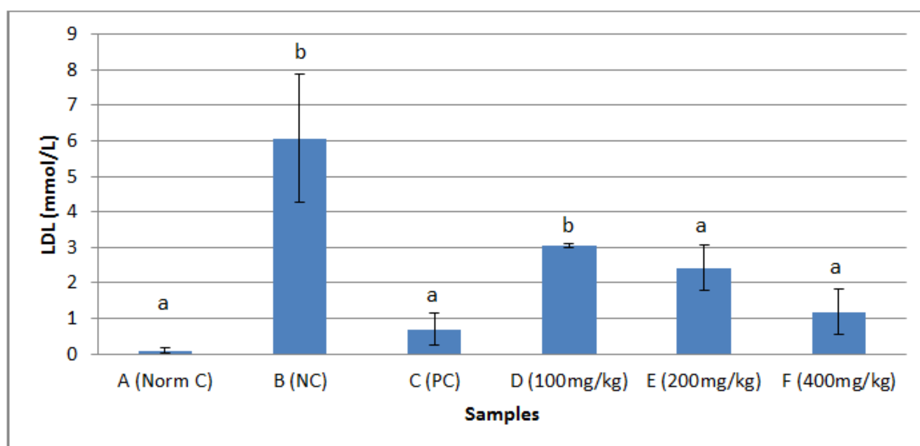

Figure 6. LDL concentration in rat's plasma treated with *MyP* water extract for 4 weeks. Results are expressed as mean ± S.D. Different superscripted letters on top of the chart indicate statistical differences ($p < 0.05$). n = 6.

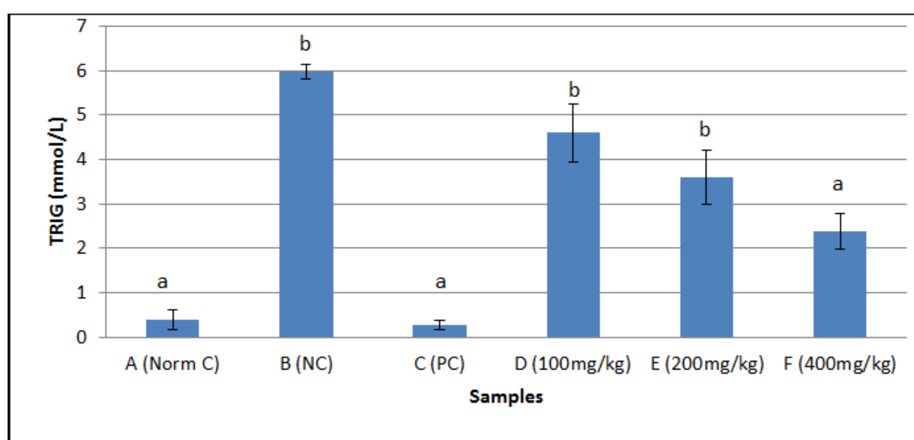

Figure 7. Triglyceride in rat's plasma treated with *MyP* water extract for 4 weeks. Results are expressed as mean ± S.D. Different superscripted letters on top of the chart indicate statistical differences ($p < 0.05$). n = 6.

Fecal Cholesterol Analyses

Faecal cholesterol contents in rats treated with *MyP* water extract for 4 weeks.

Group	Norm C	NC	PC	(100 mg/kg) *MyP* water extract	(200 mg/kg) *MyP* water extract	(400 mg/kg) *MyP* water extract
Dry weight						
g/day	5.2	6.1	5.4	6.34	5.87	5.94
Cholesterol						
(µmol/g feces)	4.17 ± 0.33a	5.21 ± 0.47a	5.27 ± 0.61a	5.29 ± 0.20a	7.91 ± 0.11b	10.44 ± 0.14c
Triglyceride						
(µmol/g feces)	4.65 ± 0.26a	5.53 ± 0.25b	5.62 ± 0.31a	6.43 ± 0.43c	8.83 ± 0.13 c	8.89 ± 0.09b

Table 1: Results are expressed as mean ± S.D. Different superscripted letters indicate statistical differences ($p < 0.05$). n = 6.

Fecal Bile Acid Analyses

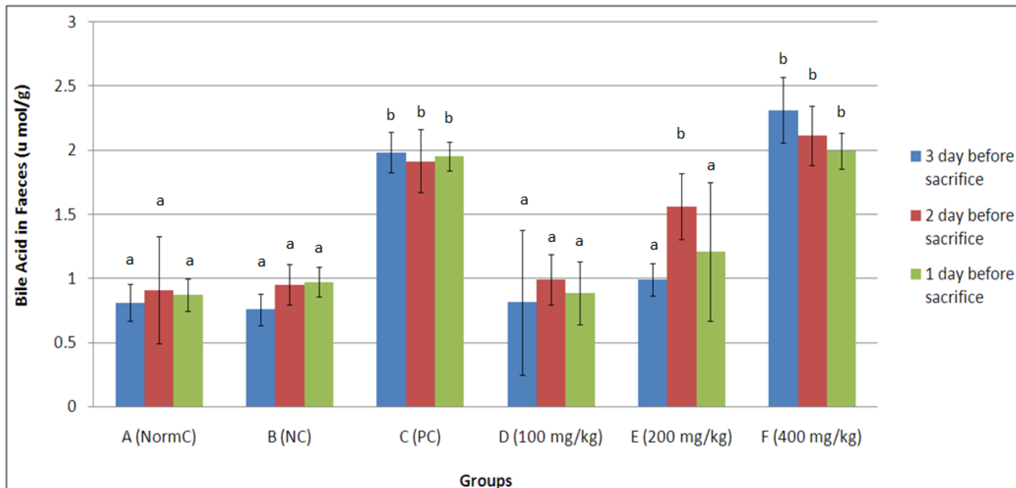

Figure 8. The concentration of total faecal bile in faeces of rats fed HCD and treated with *MyP* water extract. The values represent the means ± SD. Different superscripted letters in columns indicate statistical differences ($p < 0.05$). n = 6.

AST

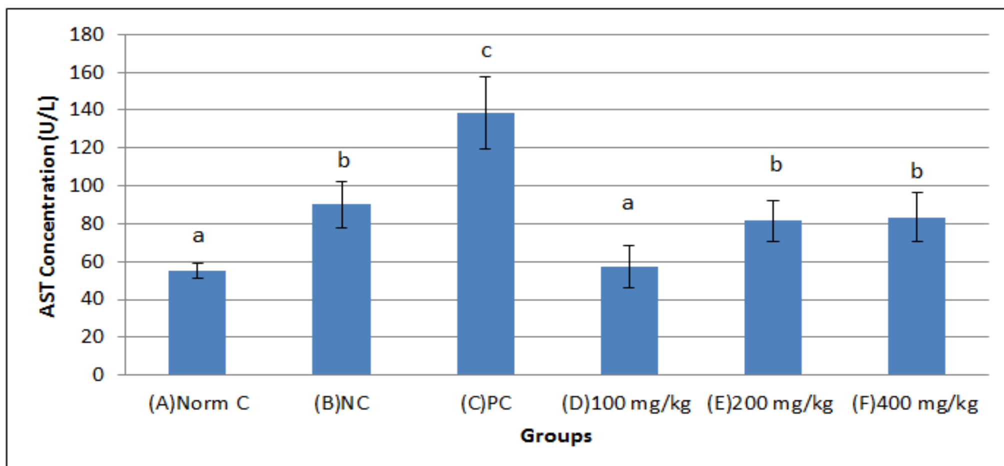

Figure 9. AST concentration in rat's plasma treated with *MyP* water extract for 4 weeks. Results are expressed as mean ± S.D. Different superscripted letters on top of the chart indicate statistical differences ($p < 0.05$). n = 6.

ALT

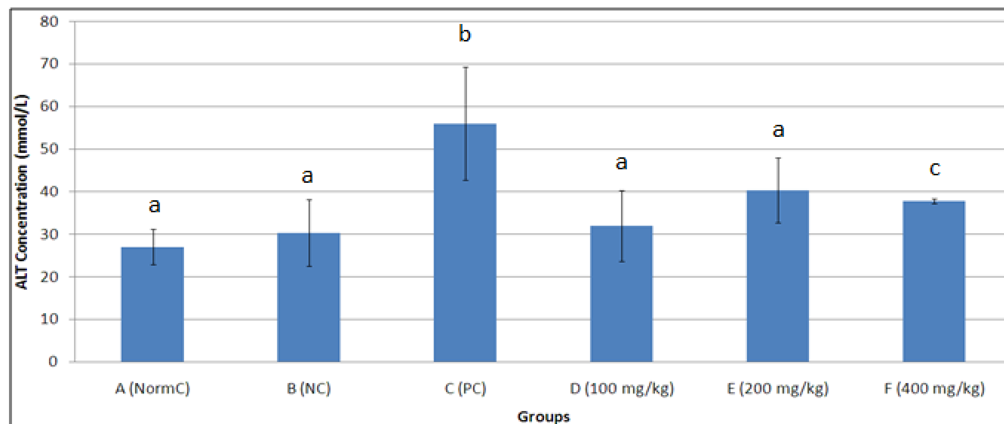

Figure 10. ALT concentration in rat's plasma treated with *MyP* water extract for 4 weeks. Results are expressed as mean ± S.D. Different superscripted letters on top of the chart indicate statistical differences ($p < 0.05$). n = 6.

ALP

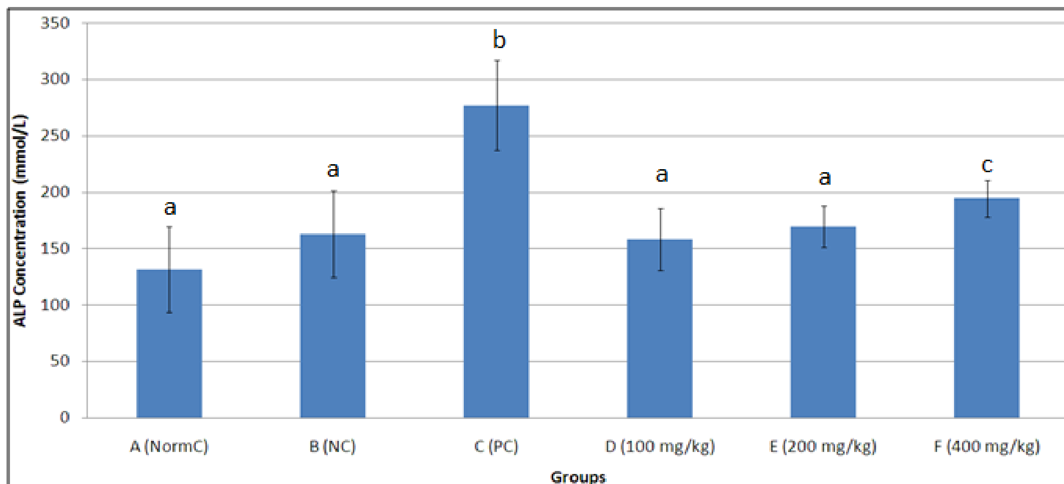

Figure 11. ALP concentration in rat's plasma treated with *MyP* water extract for 4 weeks. Results are expressed as mean ± S.D. Different superscripted letters on top of the chart indicate statistical differences ($p < 0.05$). n = 6.

Creatinine Kinase (CK)

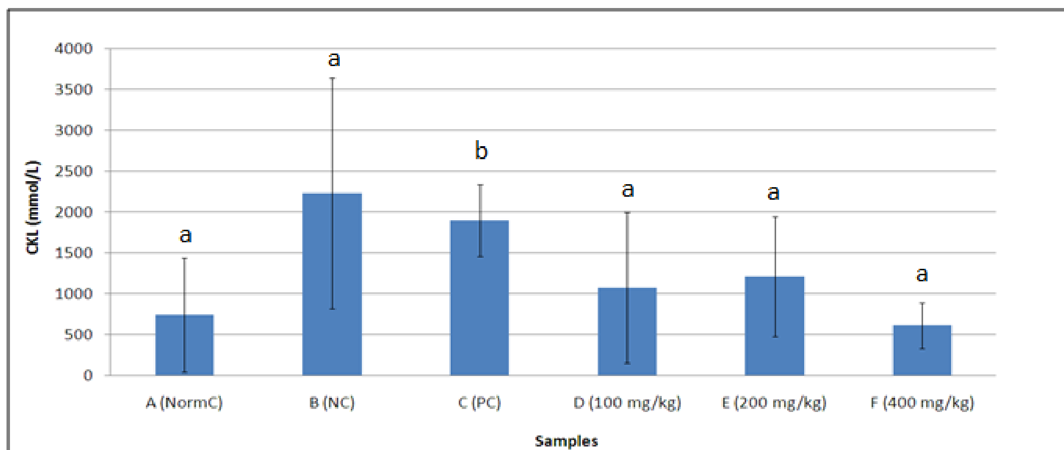

Figure 12. CK concentration in rat's plasma treated with *MyP* water extract for 4 weeks. Results are expressed as mean ± S.D. Different superscripted letters on top of the chart indicate statistical differences ($p < 0.05$). n = 6.

Urea

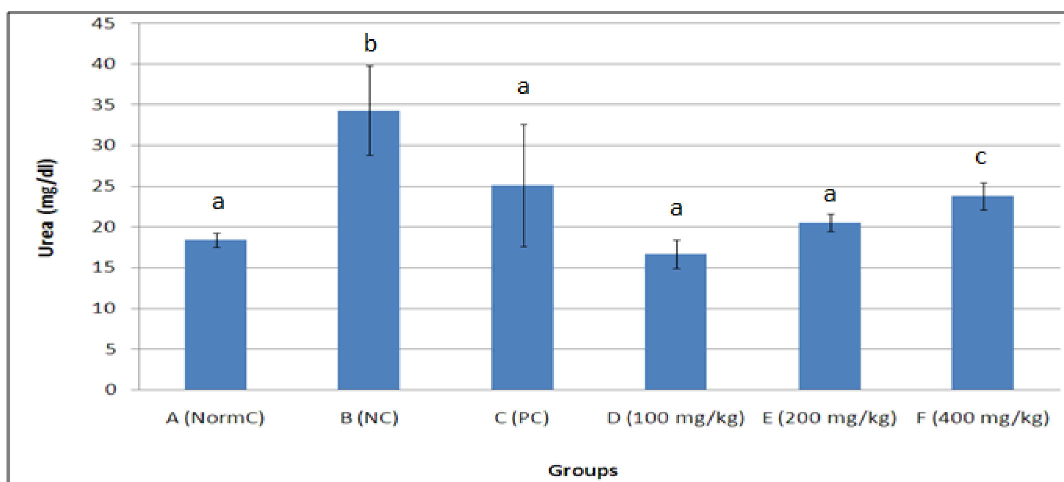

Figure 13. Urea concentration in rat's plasma treated with *MyP* water extract for 4 weeks. Results are expressed as mean ± S.D. Different superscripted letters on top of the chart indicate statistical differences ($p < 0.05$). n = 6.

Discussion

Nowadays, herbal medicines have become increasingly popular around the world (Popović *et al*., 2016). These medicines are among the most widely used as alternative treatments for many diseases (Tshitenge *et al*., 2016). However, their effectiveness, safety, and mechanism of actions largely remain unknown (Tshitenge *et al*., 2016; Snow, 2016). There are many herbs were developed as a nutraceutical and pharmaceutical product to reduce hypercholesterolemia (Chan *et al*., 2016; Mannarino *et al*., 2014).

One of the strategies that have been used to control blood cholesterol is controlling food intake containing cholesterol (Koopmans *et al*., 2015). Controlling food intake containing high cholesterol is literally decrease cholesterol absorption by intestine (Obrowsky *et al*., 2012; Vučić and Cvetković, 2016). As shown in Figure 1, there was no difference among groups on the amount of daily food intake in week 1. However, 200 mg/kg *MyP* water extract treatment showed a decline of food intake in week 2. Meanwhile, other groups did not show any significant change compared to normal control in week 2. In week 3, PC and all *MyP* water extract treatment groups showed a decrease in food intake. Though, food intake pattern was increased tremendously in week 4 in all *MyP* water extract treatment groups compared to week 3. This showed that *MyP* extract can affect the food intake of the rat. However, the impact was not as good as a positive group. Food intake of PC group was decreased and maintained in week 4. As shown in Figure 2, water intake of *MyP* water extract treated rat was decreased in week 1, week 2 and week 3 and gradually increased in week 4. Treatment of 400 mg/kg *MyP* water extract showed inclined of water intake in week 4. The normal group showed gradually decreased of water intake from week to week.

Besides that, Figure 3 showed the bodyweight of the rat was not significantly different compared to each group. Bodyweight of rat is related to animal's health (Bhardwaj *et al*., 2010). Within 4 weeks, almost all rats increase bodyweight from 250 g to 350 g. There were no tremendous decreased of bodyweight reported within 4 weeks. This indicated that *MyP* water extract was not toxic. Besides that, the levels of plasma TC were all significantly increased in the NC, *MyP* water extract and PC group compared to the normal group ($p<0.05$). There were significant differences between the normal control and positive group (Figure 4). The PC showed a higher reading of TC. However, no significant differences were exhibited between the normal group and *MyP* water extract treated group for 100 mg/kg and 200 mg/kg after 4 weeks of the experimental diet ($p<0.05$). As a reference value, the plasma TC level measured in rats fed a normal diet was 1.1 mmol/l.

The *MyP* water extract treated group exhibited a dramatic increment in the levels of plasma HDL after 4 weeks of treatment with 100 mg/kg, 200 mg/kg and 400 mg/kg ($p<0.05$) as shown in Figure 5. The HDL concentration values in the normal control and NC group were significantly lower than PC group ($p<0.05$). The supplementation of *MyP* water extract (200 mg/kg and 400 mg/kg) and Simvastatin for 4 weeks significantly increased ($p<0.05$) the levels of plasma HDL. However, the plasma HDL concentration was significantly lower in only 100 mg/kg *MyP* water extract group ($p<0.05$). This indicated that treatment of *MyP* water extract could help in HDL formation. Higher concentration of HDL in blood circulation might reduce the premature development of atherosclerosis and cardiovascular disease (Singh *et al*., 2007). Plasma levels of HDL are positively correlated with the function of anti-atherosclerosis (Lazo-Porras *et al*., 2015). In many reports, it was clearly suggested the mechanism of the anti-atherogenic effects of HDL are predominantly related to its contribution in the pathways of reverse cholesterol transport (RCT) (Mei and Atkinson, 2015).

In Figure 6, plasma LDL in the negative group showed significant higher compared to the normal group. However, PC group exhibited significant lower of LDL concentration compared to NC group ($p<0.05$). The levels of plasma LDL were significantly decreased in the 200 mg/kg and 400 mg/kg *MyP* water extract treatment groups. Meanwhile, the concentration of plasma LDL was higher in only 100 mg/kg *MyP* water extract group than the other *MyP* water extract groups. It was clearly observed that the *MyP* water extract treatment's concentration has the impact in reducing LDL concentration level in the rat's blood circulation. According to Jones *et al*., (1996), dietary cholesterol suppresses the cholesterol biosynthesis. However, dietary cholesterol slightly raises plasma concentrations (Jones, 1997). The result of LDL level increase in rat induced with HCD was supported by Jones (1997). The LDL cholesterol plays a key role in the formation of plaque atheroma. Higher concentration of LDL level in the blood circulation increases the chance of lipid peroxidation by free radical.

Besides that, treatment of 400 mg/kg *MyP* water extract was effectively decreased the TG level in the rat. Meanwhile, no significantly differences were found in the plasma TG concentrations (Figure 7) in 100 and 200 *MyP* water extract treatment group compared NC group. From the graph, it also can be seen that the TG concentration in NC group was a significant difference compared to the normal group. This occurred because of the diet that was induced to the rat containing fat. This finding was supported by the similar study that was performed by Buettner *et al*., (2006) which showed that induction of 42% fat can increase TG in the bloodstream, hepatosteatosis and accumulation of lipid droplet

in the liver. Pancreatic lipase (triacylglycerol acyl hydrolase) catalyses the digestion of dietary fat (Seyedan *et al*., 2015). It is an important lipolytic enzyme which is synthesised and secreted by the pancreas and plays a significant role in dietary TG absorption, hydrolyzing TG to monoacylglycerols and fatty acids (Seyedan *et al*., 2015). The effect of *MyP* water extract has occurred at the intestine which it decreased the digestion of fat that was ingested by the rat. The undigested fat was carried out as faecal triglyceride as shown in Table 1. The faecal triglyceride was increased when the concentration of *MyP* water extract was increased.

Moreover, the defecation process that brings out undigested cholesterol and lipid group also reducing blood cholesterol level. This occurs when the digestion and absorption process of cholesterol is interrupted by reducing pancreatic lipase enzyme activity and binding of bile acids. The rats treated with 100 mg/kg *MyP* water extract had increased faecal weight (dry) but did not significantly increase ($p<0.05$) faecal cholesterol excretion as compared to negative control. However, the faecal cholesterol excretion for the rats fed with the 200 and 400 mg/kg *MyP* water extract was significantly higher than NC group (Table 1). Correspondingly, the concentrations of faecal triglyceride in groups treated with 200 mg/kg and 400 mg/kg *MyP* water extract were significantly higher ($p<0.05$) than NC group. This indicated that the concentration of *MyP* water extract is the factor to increase the effect.

The excretion of bile acid (BA) in the group of 400 mg/kg *MyP* water extract treatment was approximately 2-fold higher than that in the normal and NC groups (Figure 8). The *MyP* water extract treatment significantly increased ($p<0.05$) the bile excretion from the intestine. These results suggested that there was an elevated of BA binding capacity in the *MyP* water extract group which can result in a decrease of the hepatic pool of BA and a reduction of LDL. However, the binding capacity of BA only effective in highest *MyP* water extract treatment group. Meanwhile, treatment for the other two concentrations did not significantly increase the BA excretion. The group that was treated with simvastatin in PC group showed the elevation of BA excretion. The higher amount of cholesterol in the faeces of the 400 mg/kg *MyP* water extract group compared to the amount of cholesterol in the faeces of the NC group can be observed in Table 1.

Bile acids act as detergents and are responsible for facilitating the absorption of dietary lipids and fat-soluble vitamins, and for maintaining cholesterol homoeostasis in the body (Jones *et al*., 2015). In enterohepatic circulation, almost 95% of bile acids are efficiently reabsorbed from the intestinal lumen and undergo a recycling mechanism to be used for maintaining of cholesterol level in the body (Gannasin *et al*., 2016). Bile acid binding property of *MyP* water extract is believed can lower down the level of bile acids reabsorption and increase the level of its excretion. Thus, hepatic synthesis of bile acids from blood cholesterol is increasing and leading to a reduction in blood cholesterol level (Gannasin *et al*., 2016). This effect may be responsible for the lowering LDL concentration observed in the *MyP* water extract group compared with the normal and negative group as can be seen in Figure 6. Besides that, bile acid also involves in triglyceride absorption at the intestinal lumen. Emulsification of lipid by bile acid is the vital process before it can be broken down by lipase enzyme. According to Sarkar *et al*., (2016) bile salts play a crucial role in lipid digestion by pushing initially adsorbed materials from the interface and permitting lipase/colipase complexes to act on the bile-coated oil droplets. This allows the process of lipid digestion which involve the breakdown of fat into small triglyceride and glycerol that can be absorbed by the enterocyte membrane. Figure 7 showed that the treatment of 400 mg/kg *MyP* water extract significantly decreased the level of triglyceride compared to NC. The undigested fat might be excreted by defecation which increased the faecal triglyceride as shown in Table 1.

Moreover, both ALT and AST transaminases are markers of hepatocyte injuries (Stolf *et al*., 2012). The results showed the plasma AST and ALT levels significantly decreased ($p <0.05$) in PC group compared to normal control group. This result was supported by the previous study done by Torcha *et al*., (2010) and Sancho *et al*., (2013). There were consistently reported that Simvastatin increases ALT concentration. ALT is a sensitive measure of liver injury, broadly used to assess potential hepatotoxicity during drug development (Moylan *et al*., 2012). Figure 9 showed that the oral administration of *MyP* water extract (200 and 400 mg/kg) showed significantly decrease ($p <0.05$) of plasma AST levels compared to PC, whereas administration of 100 mg/kg showed no significant of AST compared with normal group. Interestingly, Figure 10 showed that treatment of 100 mg/kg *MyP* water extract showed significantly decrease ($p <0.05$) in ALT (59.91±2.85 and 32.47±1.23 U/mL) levels.

The plasma levels of ALP in treated rat groups are given Fig 11 showed significantly increased in PC group. The plasma levels of ALP were significantly low in *MyP* water extract treated rats in comparison with PC group. Conversely, the plasma levels of ALP were significantly lower in the normal group. The liver enzymes, ALT, AST and ALP activities were significantly elevated in PC group in comparison with normal and NC group. These enzymes were significantly reduced in low and medium concentration of *MyP* water extract treated rats comparing with PC group.

The elevation of the plasma levels of urea and CK are considered as significant markers of renal dysfunction (Davis *et al*., 2013; Sereno *et al*., 2013). The results in Figure 12 showed significant ($p <0.05$) increase in the level of plasma CK in

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the negative group. While, after the treatment of Simvastatin the level of CK was significantly ($p < 0.05$) increased in plasma compared with the normal group. Similarly, the elevation of CK level caused by HCD was declined after the administration of the 200 mg/kg and 400 mg/kg *MyP* water extract by 28, 31 and 39% ($P < 0.05$), respectively, compared with the negative group. Besides that, Figure 13 showed the treatment of 100 mg/kg *MyP* water extract showed significantly decrease ($p < 0.05$) the urea and CK levels. Treatment of *MyP* water extract 200 mg/kg and 400 mg/kg showed significantly decrease ($p < 0.05$) the CK while treatment of 400 mg/kg showed a significant increase ($p < 0.05$) on the level of urea compared with normal control group. Generally, almost all biochemistry data showed that *MyP* water extract was not toxic.

CONCLUSION

From this in vivo animal study, MyP water extract was able to reduce LDL cholesterol concentration in blood circulation significantly compared to NC group. The standardised extract also can increase bile excretion and increase HDL concentration. Altogether, these events are signs that MyP water extract may have the potential to actively preventing hypercholesterolemia related diseases, especially atherosclerosis. Interestingly, the finding of rising of HDL level might show that MyP water extract can increase the reverse cholesterol transport process.

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