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Original Research Article

Therapeutic effects of anthocyanin-rich *Hibiscus sabdariffa* L. extract on body mass index, lipid profile and fatty liver in obese-hypercholesterolaemic rat model

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ABSTRACT

Background: *Hibiscus sabdariffa* L. or Roselle, is one of the most common flower plants cultivated worldwide. Traditionally, it is claimed to reduce weight and cholesterol level. The aim of this study was to evaluate the effects of anthocyanin-rich Roselle aqueous extract on the physical, biochemical and histological changes in obese-hypercholesterolaemic rat model.

Methods: Thirty Sprague-Dawley male rats were divided into five groups (n=6 per group). Group 1 is normal control (NC) rat that was fed with normal diet. The remaining rats (Group 2-5) were fed with commercial high-fat diet (HFD) for 6 weeks to induce obese-hypercholesterolaemic (OH) rat. After induction, the OH rats were divided into 4 groups: OH-Untreated group, OH-ARTE; OH treated with 300 mg/kg Roselle aqueous (anthocyanin-rich) and 1% trifluoroacetic acid extract (ARTE), OH-Orlistat; OH treated with 37 mg/kg orlistat and OH-Atorvastatin; OH treated with 10 mg/kg atorvastatin. After the 3 weeks intervention period, all rats were sacrificed. Body mass index, lipid profile and liver enzymes were evaluated. The liver section was subjected to hematoxylin and eosin staining for histopathological evaluation.

Results: This study showed that 3 weeks administration of ARTE significantly reduced BMI, improved levels of lipid profile and liver enzymes, and histopathological changes of liver when compared to OH-untreated rats. The results were also comparable to the standard drugs.

Conclusions: This study suggested that Roselle extract constitutes an effective and safe alternate treatment for obesity and hyperlipidaemia.

Keywords: *Hibiscus sabdariffa* L., Obesity, Hyperlipidaemia, Hypercholesterolaemia

INTRODUCTION

Obesity is a medical condition that develops from an interaction between genetic, environmental and behavioral factors. It is caused by an imbalance between energy intake and expenditure. The prevalence of obesity has been of great concern globally in both men and women worldwide. Over the years, the financial cost for the anti-obesity drugs is escalating that increases the economic burden to the health care system. Obesity is associated with excess body fat deposited in between organs, skin layers and vascular system that detrimental to health.[1] Thus, it has a strong association with the occurrence of chronic medical problems that leads to impairment of health-related quality of life. Some of the obesity-related comorbid conditions include

hypertension, atherosclerosis, non-alcoholic fatty liver disease (NAFLD), type 2 diabetes, and dyslipidaemia.[2] There is an association of obesity with hypercholesterolaemia, which further increases the cardiovascular risk.[1,3]

The approaches for obesity management are mostly dietary, exercise and behaviour modification. Current therapeutic approaches for treating obesity-related diseases cause loss of appetite and decrease absorption,[4] which can lead to more serious failure. It is generally accepted that over-the-counter herbal preparations to treat obesity have limited data documenting their efficacy or safety. However, these medicinal plants have been preferred as an alternative treatment in the management of obesity because of the lower cost, fewer side effects and well tolerated by the patient.[5-7] There are several traditional medicinal herbs have been reported to have anti-obesity and cholesterol lowering agent that include Ginseng, *Momordica charantia*, *Zingiber officinale*, *Cyphomandra betacea* and *Hibiscus sabdariffa*.[8,9] Natural bioactive compounds including phenols and flavonoids have been reported to be effective in the treatment of obesity.[10,11]

Hibiscus sabdariffa L. or Roselle is a flavonoid-rich natural product that belongs to the family of Malvaceae. This plant is one of the most common flower plants that have been cultivated globally including Malaysia, Thailand, Indonesia, India, Egypt, Nigeria and México.[7] Roselle is used to produce jam, wine and jelly. The Roselle extract was reported to reduce total cholesterol and prevent lipid peroxidation in *in vivo* study.[12] The chemical constituents of Roselle including alkaloids, anthocyanins, galactose, polysaccharides, citric acid and flavonoids.[13,14] Previous studies reported most of the chemical studies of the calyx constituents have been focus towards characterisation of anthocyanin that has been classified in flavonoid class.[15,16] Anthocyanin, a flavonoid phytopigment, is one of the extracts from this plant that is potential to reduce obesity.[17] Besides as anti-obesity, anthocyanin is known to possess antioxidant, anti-hyperlipidaemic, anti-atherosclerotic and hepato-protective properties.[18]

Studies on the association between Roselle and obesity is limited, however traditionally, it is claimed to reduce weight and cholesterol level. Therefore, this study was done to evaluate the therapeutic effect of anthocyanin-rich *Hibiscus sabdariffa* L. aqueous extract (ARTE) as anti-obesity and anti-hyperlipidaemic in obese-hypercholesterolaemia (OH) rat model.

METHODS

Plant material

The *Hibiscus sabdariffa* L. (Roselle) leaves were purchased from local supplier, Herbagus Sdn. Bhd., Pulau Pinang, Malaysia. The specimen of the plant was deposited in the Herbarium of FRIM and authenticated by certified botanist (Reference number: PID-060516-05).

Preparation of the plant extract

The extraction process was done in Herbal Medicine Research Centre at Institute of Medical Research (IMR), Malaysia. For aqueous and 1% trifluoroacetic acid (TFA) extract (ARTE) preparation, 250 g of the Roselle calyx's powdered form was extracted three times using 2500 ml distilled water (80ºC) mixed with 1% trifluoroacetic acid (TFA) and sonicated for 30 minutes at 25ºC (room temperature). The combined filtered extract was frozen overnight at -40ºC. This extract was subjected to freeze drying. After that, it was stored in amber vial at -20ºC until further used.

Identification of anthocyanin compound

The extract was quantified using centrifugal partition chromatography (CPC) and thin-layer chromatography (TLC). High performance liquid chromatography (HPLC) was also performed using the detection compound, to determine the type of compound present in the calyx extract of Roselle. This compound was then sent for liquid chromatography-mass spectrometry (LCMS) and Nuclear magnetic resonance (NMR) at Universiti Pendidikan Sultan Idris (UPSI), Malaysia for confirmation of the structure of anthocyanin.

Drugs preparation

The aqueous Roselle extract was given at 300 mg/kg, diluted with 100 mg/ml distilled water. While for atorvastatin, 10 mg/kg was diluted with 10 mg/mL of distilled water and orlistat, 37 mg/kg was diluted with 10 mg/mL of distilled water. The dosage of both drugs was extrapolated from recommended dosage in human.[19] Orlistat and atorvastatin were used in this study as the standard drugs for anti-obesity and cholesterol lowering agent respectively.

Animals and experimental design

Sprague-Dawley male rats weighing between 200 g and 250 g were randomly divided into 5 groups of 6 rats each. Rats were supplied by Animal Research and Service Centre (ARASC), Health Campus, USM Kelantan, Malaysia. Rats were caged individually in polypropylene cages at 25°C and 60-70% humidity on 12 hour light-dark cycle. Food intake and body weight were recorded. The body mass index (BMI) was calculated using the modified theory whereby rats were considered as obese if the BMI is more than 0.68 g/cm^2.[20] Meanwhile, total cholesterol levels of more than 3.05 mmol/l were considered hypercholesterolaemia.[21] Rats were euthanised using sodium pentobarbitone 100 mg/kg via intraperitoneal (IP) route.

The details of the study groups are as follows.

Normal control

It was normal control (NC) rats that were administered with normal pellets.

The other four groups were subjected for induction of obesity and hypercholesterolaemia (OH). The induction was done for 6 weeks by daily administration of commercial high-fat diet (HFD). HFD consisted of 4% cholesterol, 1% sodium cholate and 0.5% thiouracil (Altromin, Germany). After the induction, the OH rats were divided into 4 groups and treated accordingly. All treatments were administered daily for 3 weeks:

Group OH-untreated

This was the OH group that was untreated.

Group OH-ARTE

This was the OH group that treated with 300 mg/kg anthocyanin-rich Roselle aqueous and 1% TFA extract (ARTE).

Group OH-orlistat

This OH group received 37 mg/kg orlistat.

Group OH-atorvastatin

This OH group received 10 mg/kg atorvastatin.

Measurement of biochemical parameters

Levels of lipid profile (total cholesterol; TC, triglycerides; TG, low-density lipoprotein cholesterol; LDL-C and high-density lipoprotein cholesterol; HDL-C) and liver enzymes (aspartate transaminase; AST and alanine transaminase; ALT) were measured.

Histopathological study of liver

The liver was dissected out, washed in ice-cold normal saline and fixed in 10% formalin solution. Tissues were cut using a microtome blade and processed by automated tissue processing machine (semi-enclosed Benchtop Tissue Processor Leica TP 1020, Leica Microsystems NussLoch GmbH, Germany). The samples fixed in formalin were dehydrated by an ascending alcohol series ending in xylol and finally embedded in paraffin. The specimens were sectioned at a thickness of 2-3 µm and stained with hematoxylin and eosin solution.

Statistical analysis

Data were analysed using one way ANOVA followed by Bonferroni's post-hoc test. All tests were two-tailed and the significance levels were set at $p<0.05$. Data are presented as mean (standard error of mean). Data analysis and graphs were plotted using GraphPad-Prism v.6.0.1 software (GraphPad, San Diego, CA).

RESULTS

Effects of anthocyanin-rich Roselle aqueous and 1% TFA extract on BMI

Table 1 summarises the effects of different treatments on body mass index (BMI) in OH rats for 3 weeks duration. The BMI was significantly reduced in OH-ARTE and OH-orlistat groups when compared to pre-treatment level.

Table 1: Effects of different treatments on body mass index in all groups.

Groups	BMI (g/cm^2)	
	Pre-treatment	**Post-treatment**
NC	0.54 (0.01)	0.43 (0.01)
OH-untreated	0.72 (0.12)	0.70 (0.01)
OH-ARTE	0.69 (0.01)	0.46 (0.02)[a]
OH-orlistat	0.71 (0.02)	0.55 (0.02)[b]

[a]$p<0.0001$, [b]$p<0.01$ when compared to pre-treatment levels of respective groups.

Data are presented in mean (SEM).

Effects of anthocyanin-rich Roselle aqueous and 1% TFA extract on lipid profile

Table 2 summarises the effects of different treatments on lipid profile. The levels of TC, TG and LDL-C were significantly decreased but the levels of HDL-C were significantly increased in OH-ARTE and OH-Atorvastatin groups when compared to OH-untreated group.

Table 2: Effects of different treatments on lipid profile in all groups.

Groups	TC (mmol/l)		TG (mmol/l)	LDL-C (mmol/l)	HDL-C (mmol/l)
	Pre -treatment	**Post-treatment**			
NC	1.48 (0.02)	1.50 (0.03)	0.55 (0.10)[d]	0.40 (0.04)[b]	0.93 (0.03)
OH-Untreated	8.09 (0.38)	8.09 (0.13)	3.62 (1.07)	6.70 (1.99)	1.60 (0.40)
OH-ARTE	8.50 (0.53)	4.76 (0.45)[a]	0.40 (0.07)[d]	1.27 (0.11)[b]	4.57 (0.23)[c]
OH-Atorvastatin	8.52 (0.33)	2.89 (0.39)[a]	0.40 (0.10)[d]	0.87 (0.12)[b]	3.50 (0.72)[d]

[a]$p<0.0001$ when compared to pre-treatment levels; [b]$p<0.0001$, [c]$p<0.01$, [d]$p<0.05$ when compared to OH-Untreated; data are presented in mean (SEM).

Effects of anthocyanin-rich Roselle aqueous and 1% TFA extract on liver enzymes

Table 3 summarises the effects of different treatments on liver enzymes; AST and ALT. Both enzymes were significantly higher in OH-untreated group when compared to NC group. In addition, both enzymes were significantly lower in OH-ARTE, OH-Orlistat and OH-Atorvastatin groups when compared to OH-untreated group.

Table 3: Effects of different treatments on liver enymes in all groups.

Groups	AST (iu/l)	ALT (iu/l)
NC	135.00 (11.00)[a]	65.50 (6.50)[b]
OH-untreated	462.50 (7.50)	196.00 (32.00)
OH-ARTE	228.00 (0.00)[a]	100.33 (7.53)[c]
OH-orlistat	131.50 (18.50)[a]	65.50 (2.50)[b]
OH-atorvastatin	125.50 (3.50)[a]	86.00 (27.00)[c]

[a]p<0.0001, [b]p<0.01, [c]p<0.05 when compared OH-untreated; data are presented in mean (SEM).

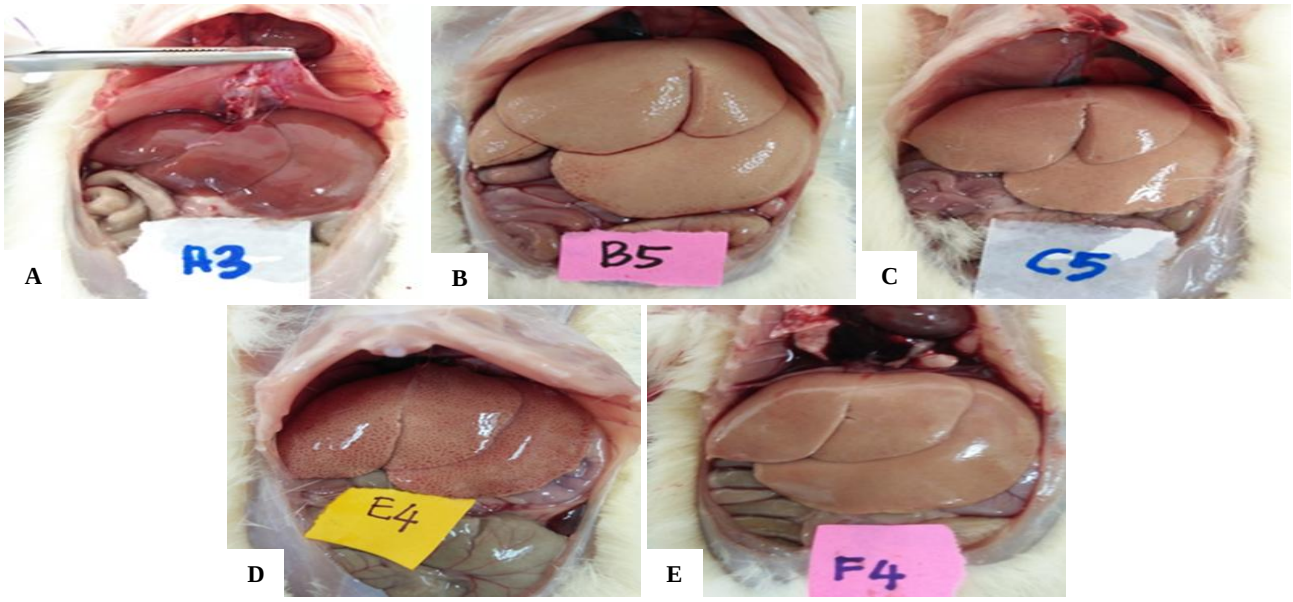

Figure 1: Gross examination of liver taken from all groups; (A) NC, (B) OH-untreated, (C) OH-ARTE, (D) OH-orlistat and (E) OH-atorvastatin; liver from NC rat appears normal in size and red in colour whereas liver from other groups are enlarged and pale yellowish in colour.

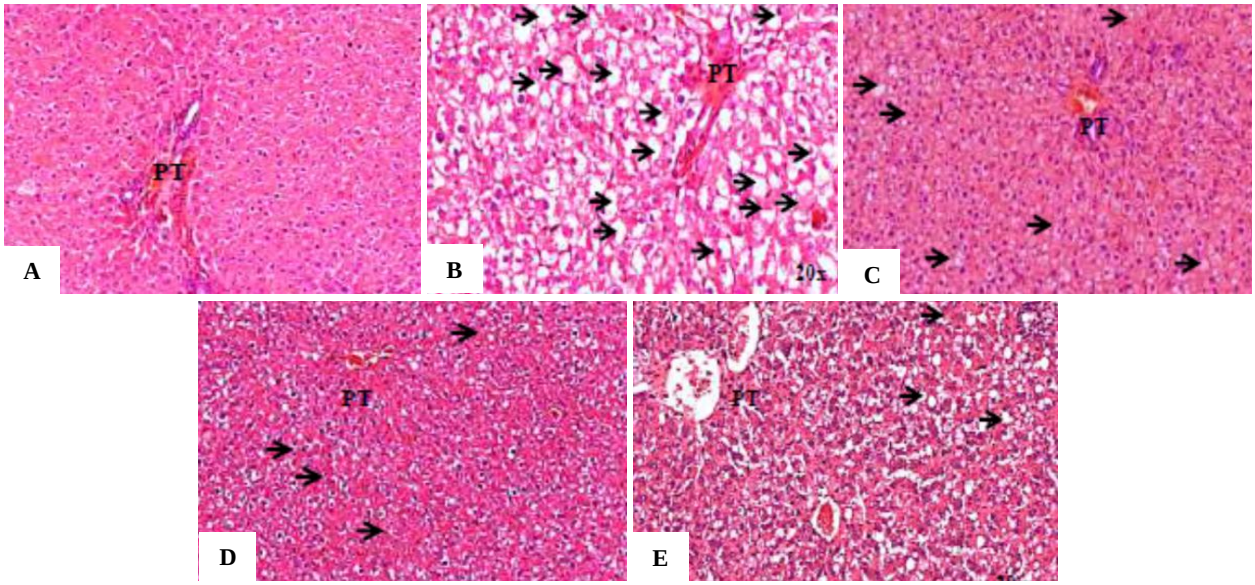

Figure 2: Light micrograph of livers section from all groups stained with hematoxylin & Eosin (20X); (A) histology of liver from NC showed normal smooth hepatic appearance; (B) presence of fatty vacuoles with foamy degeneration of hepatocytes that indicates acute fatty liver is shown in OH-untreated group; (C) livers from OH-ARTE, (D) OH-orlistat and (E) OH-atorvastatin show minimal fatty vacuoles (black arrows: fatty vacuole, PT: portal triad).

Effects of anthocyanin-rich Roselle aqueous and 1% TFA on liver

Figure 1 demonstrates gross examination of the livers. In NC rat, the liver was normal in size and red in colour (A). However, in other groups; OH-untreated, OH-ARTE, OH-orlistat and OH-atorvastatin, the livers were enlarged and pale yellowish in colour due to fat accumulation (B-E).

The liver was further evaluated by hematoxylin and eosin analysis using the light microscope. As shown in Figure 2, at 20x magnification, (A) the liver structures from NC rat showed normal smooth hepatic appearance; (B) however, the histology of OH-untreated group showed a massive steatosis with foamy degeneration of hepatocytes that indicates acute fatty liver; (C) treatment with ARTE, (D) orlistat and (E) atorvastatin showed improvement in the liver histology, minimal to moderate steatosis.

DISCUSSION

The therapeutic effect of Roselle extract against obesity, derangements of lipid profile and liver enzymes, and histopathological changes in obese-hypercholesterolamic rats was investigated in this study. The ideal anti-obesity drug would produce sustained weight loss with minimal side effects. A conventional anti-obesity may cause severe toxicity to the internal organs including the liver and kidney and do not generally produce marked or sustainable weight loss.[22] Thus, several studies have been carried out to determine the potential therapeutic effects of herbal medicine. Roselle is considered a valuable natural substance as anti-obesity, anti-hyperlipidaemic, diuresis and antihypertension.[16,21,23] In the present study, the potential of anthocyanin-rich Roselle extract as anti-obesity and anti-hyperlipidaemic were determined by measurement of the changes in body mass index, lipid profile, liver enzymes and liver histology. The extract used in the current study is a combination of anthocyanin-rich Roselle aqueous and 1% TFA extract. Anthocyanin has been reported to have good antioxidant activities.[18] 1% TFA extract was added in the extract as it was claimed to have important properties for its use in organic synthesis, and also can be used as catalyst in several reaction, such as oxidations and reductions.[24] In addition, the usage of TFA in the extraction can stabilise the extracted component.[25]

Daily administration of ARTE at 300 mg/kg daily for 3 weeks showed a significant reduction in BMI similar to previous study.[18] Although Roselle is potential as an anti-obesity, the exact mechanisms that cause reduction in BMI are not fully understood. It has been suggested that the bioactive compounds in Roselle are responsible in modulating obesity through anti-oxidant related mechanisms, inhibition of adipogenesis in adipocyte cells and inhibition of pancreatic lipase.[26] They have proposed that the development of obesity is prevented by decreasing hyperplasia and hypertrophy of white adipocyte cells. Thus, Roselle extracts play a critical role in preventing adipogenesis and reducing accumulation of cytoplasmic lipid droplets. Roselle extracts may also inhibit the alpha-amylase, at the same time blocking sugars and starch absorption in the body that leads to weight loss.[27] Anthocyanin intake has been reported to have potential therapeutic effects in reducing the risk of developing obesity.[17] Interestingly, this effect was comparable to the treatment with orlistat. Orlistat is a standard drug to treat obesity and has been shown to have protective effects against obesity in high fat diet-induced obese rats.[28] Orlistat is the only medication that was approved by Food Drug Administration (FDA) for weight management in adolescents with obesity.[29]

Diet and exercise have been recognised as non pharmacological approach in the management of hypercholesterolaemia. However, a non pharmacological approach is not sufficient to lower the cholesterol level for most of the people. Hence, traditional herbal medicine could be one of the alternatives to reduce cholesterol levels. Administration of 300 mg/kg ARTE significantly lowered blood cholesterol in this study. Thus, this study provides another evidence that ARTE is potential to reduce cholesterol levels of HFD-induced hypercholesterolaemic rats. The results were comparable to atorvastatin group, a positive control for reduction of blood cholesterol level, similar to previous study.[30] Lipids that contain in the diet are absorbed into the bloodstream. It will then digested and stored in the liver and adipose tissue as TG.[31] Besides lowering the levels of total cholesterol, ARTE has also significantly lowered TG and LDL-C when compared to OH-Untreated group, which is consistent with previous study.[32] Roselle extract has been suggested to reduce the number of oxidised LDL by trapping the reactive oxygen species (ROS) in the plasma and interstitial fluid.[33] We have also shown that in OH-ARTE group, HDL was significantly increased when compared with OH-untreated group. Elevated TC, TG and LDL-C and decreased HDL-C have been implicated in the pathogenesis of cardiovascular disease. Increased HDL-C and decreased LDL-C reduced the risk of obesity and its complications.

Obesity is known to affect the liver function.[31] Serum ALT and AST levels have been used to determine the activity of hepatocytes.[34] This study showed increased serum AST and ALT levels in OH-Untreated rats that may suggest liver damage. When liver cells are damaged, both enzymes leak out into the bloodstream and their levels in the blood become higher than normal. Increased of liver enzymes may cause toxicity, inflammation and tissue trauma.[31] This study demonstrated the administration of ARTE decreased both of the enzymes similar to the previous report.[35] The authors proposed the presence of protocatechuric acid and anthocyanins isolated from Roselle have a protective effect against tart butyl hydroperoxide induced hepatic toxicity in rats.

Obesity is associated with increased adipose tissue and fat accumulation and liver damage.[2] Fatty liver disease is often associated with obesity.[36] We have shown that consumption of high fat diet in 6 weeks leads to fatty liver. Liver is the main organ in lipid metabolism and storage, thus changes that occur in the liver was evaluated in this study using H&E staining. In healthy normal rats, the liver is red in colour and histologically demonstrated smooth hepatic appearance. Meanwhile, in OH-untreated rat, the liver was enlarged and pale-yellowish in colour due to fat accumulation. A significant steatosis with foamy degeneration of hepatocytes was observed histologically. It is suggested that high fat diet caused acute fatty liver, an accumulation of fat cells, similar to previous study on hamster.[31] Treatment with ARTE markedly improved the liver structure to near-normal morphology, with less fat cell accumulation. Roselle exhibits multiple biological activities in human health such as antioxidant and hepatoprotective. These improvements might be attributed to the effect of anthocyanin in reducing hepatic lipid accumulation.[37] The improvement in the levels of lipid profile and liver enzymes may contribute to the improvement in the liver histology.

CONCLUSION

The present study demonstrated that daily feeding with HFD for 6 weeks was able to induce obesity and high cholesterol in Sprague Dawley rats. Thus, the current HFD regime was successful to develop an animal model of obese-hypercholesterolaemic rat. Administration of ARTE improves the BMI, lipid profile, liver enzymes and fatty liver. Taken into account of these findings, Roselle has potential as anti-obesity and anti-hyperlipidaemic agents. The effects are possibly due to the antioxidant effects of anthocyanin.

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