ISSN: 13434292
Volume 137, Issue 02, December, 2019

Study on the interaction between polymeric medical devices and pharmaceuticals –effects of characteristics of pharmaceutics on environmental stress cracking on polymeric medical devices induced by their interaction

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ABSTRACT— Defatted dabai peel contains a high amount of anthocyanin. Anthocyanins are known to prevent several types of disease, including cardiovascular-related complications. This study aimed to describe the effects of different doses of defatted dabai peel extract by histopathological analyses on lesions in the liver, kidney, heart and aorta. Histopathology methods were applied to determine the protective effects of defatted dabai peel extracts against hypercholesterolemia-induced oxidative damages to animal organs. Haematoxylin and eosin staining was applied for histopathology examination for liver, kidney, heart and aorta. Data showed that a high dose of defatted dabai extract (3000 mg per day) applied to hypercholesterolemic rabbits for eight weeks had mild protective effect, especially reducing the severity of hepatic fibrosis and steatosis of the renal medulla. The high dose of extract supplementation also reduced inflammation of aorta and formation of atherosclerosis plaque in the cell wall of right ventricle of the heart. The high dose of defatted dabai peel extract could be a protective agent against oxidative stress.

KEYWORDS: Dabai, Extract, Histopathology, Hypercholesterolemic, Organ

1. INTRODUCTION

Dabai (Canarium odontophyllum) is a high-fat fruit that shares some similar characteristics to olive and palm fruits. The trees of dabai are farmed in Borneo Island, especially Sarawak for its fruit. Dabai fruit has 24% fat, [1] and the fat is rich in carotenoids and phenolic compounds. Dabai peel has high phenolic content, especially anthocyanins. Defatted dabai peel is a waste produced from dabai oil extraction. It has high total anthocyanins (80 mg/g extract) while the defatted dabai pulp is rich in phenolic compounds such as flavonoids and saponin derivatives [2]. A previous study found that defatted dabai pulp powder significantly improved the lipid profile and atherosclerosis lesion area in hypercholesterolemic rabbits after eight weeks of supplementation [3]. In vitro protective effects of defatted dabai extracts have been reported by [4] However, no documentation on toxicity effect of the anthocyanin- rich defatted dabai, except for liver function tests determined based on plasma ALT, AST and GGT. Besides, [5] reported toxicity effect in male Charles Foster rats fed with a very high dose [2000 mg/kg body weight (BW)] of anthocyanin-rich extract of dried calyx of Hibiscus sabdariffa, but not at a lower dose (300 mg/kg BW). The toxic effect was preceded by severe weight loss with no significant histopathological changes. Moreover, [6] also reported that anthocyanin-rich extracts of grape and berries have vascular protective effects. On the basis of the protective effects of anthocyanin-rich extract of defatted dabai against oxidative damage, especially defatted dabai peel, [4] the anthocyanin-rich extract of defatted dabai peel of different dosages should be further examined for protective effects against oxidative damages in an animal model using histopathological method. Histopathological examination is one of the important tools that can be applied to determine

protective effects of plant extracts in liver and kidney tissues.

2. CASE REPORT

A total of 36 male New Zealand white rabbits at age of 8–10 weeks were acclimatised for two weeks in the ambient temperature of 28˚C. The initial body weights of the rabbits (1.5–1.7 kg) were recorded and caged individually. After two weeks, the rabbits were randomly distributed into six groups (n=6 per group) on basic diet in individual cages. Rabbits in normal diet group (NC) were fed on normal chow while rabbits in hypercholesterolemic group (PC) were given normal basal diet containing 0.5% cholesterol [7]. The other rabbits in hypercholesterolemic groups were supplemented with statin (HS), low (HL), moderate (HM), and high (HH) doses of defatted dabai peel extract. All rabbits received 20 g of the respective diets and supplemented ad libitum. HS group received 10 mg of simvastatin per kg BW daily. HL, HM and HH groups were supplemented with 1000, 2000 and 3000 mg of defatted dabai peel extract per day (80 mg of total anthocyanin equivalent in 1000 mg extract) throughout eight weeks of the experimental period. Preparation of high cholesterol diet was adapted based on the procedure described by Shimizu et al.8. The experimental protocol was approved by the Animal Care and Use Committee of the Faculty of Medicine and Health Sciences, Universiti Putra Malaysia (Approval no.: UPM/FPSK/PADS/BR-UUH/00385). At the end of week 8, all survived rabbits (3-5 rabbits per group) were sacrificed. Livers, kidneys, hearts and aortas of all the rabbits were collected. Whole organs were dissected except for the aorta that was dissected from the region between its origin and bifurcation into the iliac arteries. Rabbit organs including aortas were washed with normal saline, and fat residue on the outer surface was removed. All organs and aortas of three rabbits in each group (n=3) were fixed in 10% formalin and stored at 4˚C before histopathology study. The organs of other rabbits were kept at -40˚C for other analyses.

3. RESULTS AND DISCUSSION

Micrographs obtained from H&E staining of liver and kidney tissues are shown in Figures 1 and 2, respectively. Microscopic examination of the liver showed that rabbits in PC group had markedly expanded hepatocyte cytoplasm. Such observation has been reported by [10] in fatty liver diseases, and has been named as microvesicular steatosis. The parenchyma was diffusely involved, and slight necrosis was indistinctly seen. Inflammation that coupled with fibre extension and accumulation of collagen signified fibrosis of liver tissues in the hypercholesterolemic rabbits. This observation is in line with a study by [11]. that typical lesions of steatohepatitis were observed in rabbits supplemented with 0.5% cholesterol. Figure 1 shows the normal morphology of hepatocytes histological specimen of the rabbit (Figure 1A), hepatic fibrosis (Figure 1B), lesser in the degree of hepatic fibrosis in the specimens of the defatted dabai peel extract (three doses) (Figures 1C–E), and statin (Figure 1F) supplemented to hypercholesterolemic rabbits. The clear hepatic fibrosis was observed, but there was no heavy fat infiltration observed as the biomarker of fatty liver in all hypercholesterolemic groups especially PC group. Minor improvement in hepatic fibrosis was clearly seen in HL and HS, and a remarkable improvement in the hepatic fibrosis was observed in HM and HH. In this study, the used of statin showed no great improvement in liver fibrosis. Besides, [12] reported the possible limitation in the use of statin and it is hepatotoxic. Supplementation of 1000 mg of defatted dabai peel extract per day to the hypercholesterolemic rabbits resulted in improvement of the plaque deposition (reduced to 5 mm or less) in two out of three rabbits. The 3000 mg per day of extract supplementation had also further reduced the thickness of atherosclerosis plaque with one of the rabbits without deposition of atherosclerosis plaque. Similarly, defatted dabai peel extract had a mild reduction in the incidence of atherosclerosis plaque formation in the right ventricle of the hypercholesterolemic rabbits. The thickness of atherosclerosis plaques was not measured due to only partial deposition of the plaque in certain areas of the heart ventricle. The increment in the dose of defatted dabai peel extract had further decreased the thickness of the atherosclerosis plaque. Supplementation of a high dose of the defatted dabai

peel extract (3000 mg per day) and 1.2 mg simvastatin per kg BW per day had inhibited the formation of atherosclerosis plaque in the inner cell wall of aortas in one and two of the hypercholesterolemic rabbits, respectively.

Oxidative stress mainly causes atherosclerosis plaque formation. In the high oxidative condition, reactive oxygen and nitrogen species cause inflammation to the inner cell wall of aorta. Inflammatory plaque of the aorta observed in this study is shown in Figure 3F, where the inflammatory plaque can be seen as purplish-blue colour. The severity of the plaque formation was categorised into mild and severe, where the PC, HM and HL groups fall into the severe category. Deposition of a fatty streak was also observed in the right ventricle of the rabbit's heart (Figure 3D). The supplemented anthocyanin-rich extract played an important role in scavenging free radicals, thus reduced oxidative stress.

4. CONCLUSION

Protective effects of the anthocyanin-rich extract of defatted dabai peel at three different doses supplemented to hypercholesterolemic rabbits were observed. High dose (3000 mg per day) of the extract supplementation showed better protective effects than the other doses in reducing high cholesterol-induced liver fibrosis, but not for steatosis of the renal medulla. Minor improvements for the lesions of inner cell wall of the aorta and the right ventricle of the heart of the hypercholesterolemic rabbits were also observed. Although minor improvement in the lesions was observed for the treatment groups, the protective effects of defatted dabai peel extracts against diseases are not conclusive. Due to the protective effects were tested based on a single assay, the use of a few types of staining is recommended for future studies to confirm further the protective effect of anthocyanin-rich extract in the prevention of diseases. Among different doses of the extract studied, we suggest the use of a high dose of anthocyanin-rich extract (3000 mg per day) for supplementation.

5. ACKNOWLEDGEMENT

This study was funded by the Research University Grant Scheme (04-02-10-0921RU, 9192600) from the Research Management Centre, Universiti Putra Malaysia and the Science Fund from the Ministry of Agriculture & Agro-Based Industry Malaysia (05-01-04-SF1137, 5450555). We would like to extend our big thank to the laboratory staffs of Department of Pathology, Faculty of Medicine and Health Sciences for contributing to the success of this study.

6. REFERENCES

[1] Chew LY, Prasad KN, Amin I, et al. Nutritional composition and antioxidant properties of Canarium odontophyllum Miq. (dabai) fruits. J Food Compos Anal 2011;24(4):670-77.

[2] Khoo HE, Azrina A, Ismail A, et al. Solid phase extraction of antioxidative properties in the waste of dabai oil extraction. Molecules 2012;17(8):9754-73.

[3] Shakirin FH, Azlan A, Ismail A, et al. Anti-atherosclerotic effect of Canarium odontophyllum Miq. fruit parts in rabbits fed high cholesterol diet. Evid Based Complement Alternat Med 2012;2012:Art ID 838604.

[4] Khoo HE, Azlan A, Ismail A, et al. Inhibition of oxidative stress and lipid peroxidation by anthocyanins from defatted Canarium odontophyllum pericarp and peel using in vitro bioassays. PloS One 2014;9(1):e81447.

[5] Fakeye TO, Pal A, Bawankule DU, et al. Toxic effects of oral administration of extracts of dried calyx of Hibiscus sabdariffa Linn. (Malvaceae). Phytother Res 2009;23(3):412-16.

[6] Schini-Kerth VB, Étienne-Selloum N, Chataigneau T, et al. Vascular protection by natural product-derived polyphenols: in vitro and in vivo evidence. Planta Med 2011;77(11):1161-67.

[7] Nurulhuda MH, Azlan A, Ismail A, et al. Cholesterol-lowering and atherosclerosis inhibitory effect of Sibu olive in cholesterol fed-rabbit. Asian J Biochem 2012;7(2):80-9.

[8] Shimizu T, Nakai K, Morimoto Y, et al. Simple rabbit model of vulnerable atherosclerotic plaque. Neurol Med Chir 2009;49(8):327-32.

[9] Liu JY, Chen CC, Wang WH, et al. The protective effects of Hibiscus sabdariffa extract on CCl4-induced liver fibrosis in rats. Food Chem Toxicol 2006;44(3):336-43.

[10] Brunt EM. Pathology of fatty liver disease. Modern Pathol 2007;20: S40-S48.

[11] Arhan M, Öztürk HS, Turhan N, et al. Hepatic oxidant/antioxidant status in cholesterol-fed rabbits: effects of garlic extract. Hepatol Res 2009;39(1):70-7.

[12] Choe CC, Kim HS, Jeong TS, et al. Naringin has an antiatherogenic effect with the inhibition of intercellular adhesion molecule-1 in hypercholesterolemic rabbits. J Cardiovasc Pharmacol 2001;38(6):947-55.