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RESEARCH ARTICLE

Role of PPAR-A Signaling Pathway on the Protection of Antituberculosis-Induced Liver Damage by Purple Sweet Potato Extract

I Gusti Ayu Artini[1*], I Made Jawi[1], I Nyoman Mantik Astawa[2], I Wayan Putu Sutirta Yasa[3]

1. *Department of Pharmacology and Therapy, Faculty of Medicine, Udayana University.*
2. *Faculty of Veterinary Medicine, Udayana University.*
3. *Department of Clinical Pathology, Faculty of Medicine, Udayana University.*

***Corresponding Author:** *I Gusti Ayu Artini*

Abstract

Background: *Peroxisome proliferator activated receptor*-α (PPAR-α) participates in protecting the liver from many cases of liver damage. It is involved in reducing lipid accumulation, oxidative stress and inflammation. Purple sweet potato extract is one of herbal extract that posses hepatoprotector activity related to its antioxidant and antiinflammatory action. Its hepatoprotective effect on isoniazid and rifampicin induced hepatic injury has not been established yet, as well as its effect on PPAR-α expression and activation in the liver. The aim of this research was to investigate the hepatoprotective effect of purple sweet potato extract on hepatic injury induced by isoniazid and rifampicin, as well as the effect of purple sweet potato on PPAR-α expression and activation in the liver. Methods: The study design was randomized post test only control group design. We included male Wistar rats, age 8-12 weeks, weight 180-220 gram (divided into 3 groups: control, intervention and normal group). The extraction was performed with masseration technique. PPAR-α expression was measured with ELISA; activated PPAR-α was detected using immunohistochemistry; ALT level was measured with spectrophotometry. Data were analyzed using One Way Anova test, continued with Post Hoc test. Results: The result represented that PPAR-α expression in intervention group was significantly higher than control (1.344±0.249 *vs.* 0.587±0.306; p<0.001). The activated PPAR-α expression in intervention group was also significantly higher than control (2.736±0.628 *vs.* 1.903±0.397; p<0.001). ALT level in intervention group was significantly lower than control (24.169±1.080 *vs.* 39.272±0.513; p<0.001).Conclusion: In conclusions, hepatic damage induced by INH-RIH could be prevented by purple sweet potato ethanolic extract administration which potentially improved liver function through increased PPAR-α expression and activation.

Keywords: *Antituberculosis; Hepatoprotective; Ppar; Purple sweet potato.*

Introduction

Peroxisome proliferator activated receptor-α (PPAR-α) posses many protective effects on several liver diseases, regarding its role on reducing lipid accumulation, oxidative stress and inflammation. As intracellular receptor and also transcription factor, PPAR-α is involved in decreasing inflammation and oxidative stress, whether by interacting with PPAR response element (PPRE) of target gene or by affecting other transcription factor such as NFκB. Several genes that encode proteins for fatty acid metabolism (especially beta oxidation), antiinflammatory and antioxidant proteins may serve as target genes for PPAR-α. The decrease of PPAR-α expression may induce liver damage through increasing lipid accumulation, inflammation and oxidative stress[1-3].The role of PPAR-α on the pathogenesis of hepatic injury induced by isoniazid is crucial. An *in vivo* study investigated the liver toxicity of isoniazid had demonstrated that induction with isoniazid and lipopolysaccharide led to reduced expression of PPAR-α in the liver. This would cause lipid accumulation and inflammation, thus would result in hepatic damage which

represented histopathological features such as steatosis, necrosis, and infiltration of inflammatory cells[4].Several researches had proved that many anthocyanin-rich plant extracts might significantly induce PPAR-γ expression in the liver [5-6]. Previously, the role of PPAR-α in liver protection was mostly associated with reducing lipid accumulation.

The hepatoprotective effect of purple sweet potato extract as one of anthocyanin-rich plant extract was mostly related to its antioxidant and antiinflammatory action[7]. Moreover, antioxidant effect of purple sweet potato extract mostly related to activation of Nrf2, whereas antiinflammatory action of purple sweet potato extract was mostly associated with inhibition of NFκB activity.

To date, there has been no evidence yet reported the effect of purple sweet potato extract on PPAR-α expression and activation in the liver. Therefore, we conduct this study to investigate the effect of purple sweet potato ethanolic extract on liver PPAR-α expression and activation on isoniazid and rifampicin-induced rats. By conducting this research we could explore more about the role of PPAR-α in the hepatoprotective efect of purple sweet potato extract.

Methods

Study Design

Our research was an experimental study with randomized post test only control group design. We conducted this study at Integrated Biomedical Laboratory of Medical Faculty, Udayana University. The study protocol had been approved by the Animals Ethics Committee of Veterinary Faculty, Udayana University, Indonesia. We included twenty seven male rats (*Rattus norvegicus*), strain Wistar, age eight to twelve weeks, and weight 180-220 grams. Rats were randomly divided into three groups (each contain nine rats), namely:

- Control Group: Induced with INH-RIF combination, but only received aquabidest for intervention.
- Intervention Group: Induced with INH-RIF combination and also received purple sweet potato extract as intervention.
- Normal Group: Neither induced with INH-RIF combination nor received extract for intervention (only received aquabidest).

Induction of Liver Injury

For inducing liver damage, we applied 100 mg of INH-RIF combination/200 grams rat/day through intragastric route. We used a fixed combination of isoniazid and rifampicin in oral dosage form (tablet) which consists of 150 mg isoniazid and 150 mg rifampicin. We prepared the combined INH-RIF suspension by diluting the combination tablet in aquabidest for oral administration. Induction of liver injury was performed by administering the INH-RIF suspension for 28 consecutive days or four consecutive weeks [8].During the induction, rats were also given foods and water ad libitum.

Administration of Purple Sweet Potato Extract

For extraction of purple sweet potato, we used masseration technique with 95% ethanol. The purple sweet potato plants were locally derived from Mambang Village, Tabanan, Bali, Indonesia. Purple sweet potato extract was administered to intervention group as many as 200 mg/200 grams rat/day intragastrically.

The intervention with purple sweet potato extract was started from 7 days before INH-RIF induction, and continuedly applied during the INH-RIF induction. On day 29, all rats were sacrificed. The blood and liver samples were collected for investigating several biomarkers for hepatic injury.

Assessment of Biomarkers for Hepatic Injury

We assessed the total PPAR-α expression in the liver by means of enzyme linked immunosorbent assay (ELISA) technique. For analysing the activated PPAR-α expression in liver, we performed immunohistochemistry (IHC) staining which then quantified by means of analysis software. For IHC staining we used anti-PPAR alpha phospho S12. The serum ALT concentration was measured with spectrophotometry technique.

Data Analysis

The results were then analyzed with One Way Anova test for comparing the PPAR-α and ALT levels among three groups. The p-value below 0.05 was considered as statistically significant.

Results

PPAR-α Expression

The total and activated PPAR-α expression in the liver on rats were represented in Table 1. The total PPAR-α expression in control group demonstrated a significantly lower number than in normal group, whereas the total PPAR-α expression in intervention group revealed a significantly higher number than in control group.

This represented that combined INH-RIF induction resulted in a decrease of total PPAR-α expression. Contrary, intervention with purple sweet potato ethanolic extract had been proved to increase the total PPAR-α expression in the liver. Post hoc analysis also revealed similar findings (Table 2). Similar findings were also reported for the activated PPAR-α expression. The activated PPAR-α expression in control group also demonstrated a significantly lower number than in normal group, whereas the activated PPAR-α expression in intervention group stated a significantly higher number than in control group. This represented that combined INH-RIF induction resulted in a reduction of activated PPAR-α expression, while intervention with purple sweet potato ethanolic extract had shown to protect the liver by increasing the activation of PPAR-α.

The immunohistochemical feature of activated PPAR-α expression in all groups were showed on Figure 1. Immunohistochemical feature had clearly showed that brown colour was widely distributed on the intervention and normal group. It revealed that more activated PPAR-α were expressed and bind to its antibody in the liver cells on the intervention and normal group compare to the control group.

Table 1: The total and activated PPAR-α expression, and ALT concentration in the liver

Biomarkers	Control Group (Mean ± SD)	Intervention Group (Mean ± SD)	Normal Group (Mean ± SD)	p-value
Total PPAR-α (ng/mL)	0.587 ± 0.306	1.344 ± 0.249	1.045 ± 0.342	<0.001*
Activated PPAR-α (ng/mL)	1.903±0.397	2.736±0.628	2.942±0.534	<0.001*
ALT (U/L)	39.272 ± 0.513	24.169 ± 1.080	18.987 ± 0.379	<0.001*

* represented statistically significant

Table 2: Post Hoc analysis for PPAR-α expression in the liver

Compared group	Mean difference	p-value	95%CI	
			Lower	Upper
Normal vs. Control	0.458	0.004*	0.165	0.751
Normal vs. Intervention	-0.299	0.050	-0.593	-0.006
Control vs. Intervention	-0.758	<0.001*	-1.051	-0.464

* represented statistically significant

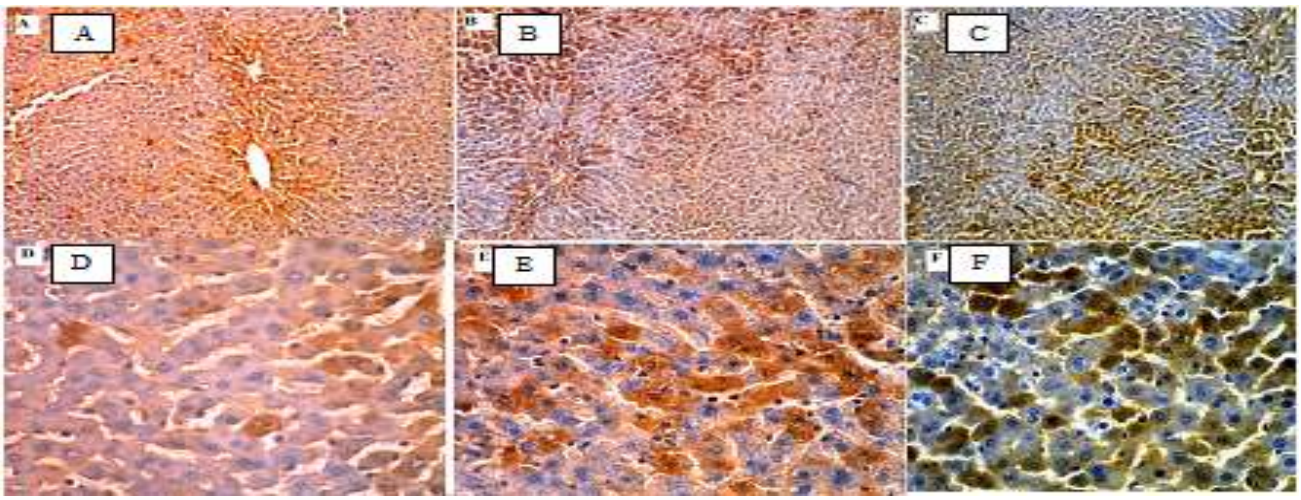

Figure 1: The activated PPAR-α expression in the liver
A and D= control group; B and E= intervention group; C and F= normal group
A,B,C= 100x magnification; D,E,F= 400x magnification

Discussions

PPAR-α (NR1C1) is one of PPAR isoforms which largely expressed in some tissues with high catabolic capacity including liver, intestine, kidney, heart, muscles, brown adipose tissuses and inflammatory cells. Like other isoforms of PPAR, PPAR-α is a nuclear

receptor that mostly functioned as transcription factor. Human PPAR-α is encoded by PPAR-α gene which located at chromosome 22. Ligands for PPAR-α are lipophillic molecules including many forms of fatty acids and their metabolites, such as arachidonic acid, prostaglandin, leucotriene etc.

Specifically, endogenous ligands for PPAR-α are linoleic acid, linolenic acid, arachidonic acid, docosahexaenoic acid, eicosapentaenoic acid and eicosanoid. Exogenous or synthetic ligand for PPAR-α which had been widely available in the market is fibrates [1-3, 9].PPAR-α had been proved to be associated with several crucial process in human body, namely proliferation, lipid catabolism, inflammation and oxidative stress.

Specifically, PPAR-α might potentially protect our body from lipid accumulation, inflammation and oxidative stress. These role of PPAR-α might be produced through several mechanisms, namely direct and indirect mechanism. PPAR-α might result in transactivation or transrepression of target gene. [1-2, 10].

Direct mechanism requires direct binding of PPAR-α to its response element (PPAR-α response element) which presents on the promoter region of several target genes. Once a ligand binds to PPAR-α, it will subsequently cause conformational changes on the receptor which then create a heterodimer complex with retinoid-x-receptor (RXR). These changes would lead to interaction with coactivator mollecule (such as p300, CREB binding protein, or steroid receptor coactivator).

Thus, this complex will result in chromatine structure remodelling through histone acetylation. It will open the chromatine which allow this complex to interact with PPRE. PPAR-α is located predominantly inside the nucleus. After synthesized in ribosome, PPAR-α is transported into the nucleus by importin α [1-3,10-11].

Target genes that might be affected by PPAR-α are genes that encode enzymes for fatty acid catabolism or ß-oxidation process, antiinflammatory proteins and antioxidant proteins. These may include acyl coA oxidase, acyl coA dehydrogenase, acyl coA synthetase, superoxide dismutase, catalase, heme oxygenase-1, etc [2, 12].

Indirect mechanism commonly related to its role on other transcription factors such as NFκB. Once the receptor is activated, it will interact with p65 subunit of NFκB protein, thus will inhibit NFκB action on promoting transcription of many proinflammatory genes. PPAR-α may also promote transcription of IκB protein that will subsequently reduce IκB degradation.

IκB is an inhibitory protein which normally binds to NFκB. When any stimulation for inflammation occurs, it will induce the phosphorylation of IκB by IκK (IκB kinase), leads to IκB release from its binding to NFκB. IκB will then be degraded by proteasome through ubiquitination process, while NFκB will activate transcription of many proinflammatory genes and induce inflammatory reaction [1-2].

Other pathways may also be affected by PPAR-α are MAPK (Erk 1/2), Akt and AMPK. PPAR-α may inhibit the phosphorylation of Erk 1/2, as well as promote the phosphorylation of GSK3ß (through activation of Akt) and phosphorylation of AMPK. Phosphorylation of GSK3ß will inactivate GSK3ß and inhibit NFκB action. AMPK phosphorylations will results in inactivation of coactivator p300 (through activation of SIRT1) thus may decrease acetylation of p65 (subunit of NFκB).

This will subsequently inhibit transcription of many proinflammatory proteins which activated by NFκB (such as TNF-α, IL-6, IL-1ß, cyclooxygenase, phospholipase A2, iNOS, VCAM-1, ICAM-1, etc.)[1-2].PPAR-α activation might be resulted from ligand-dependent or ligand-independent transcription activation. Ligand-dependent transcription activation is related to ligand binding to specific site in the PPAR-α structure, namely carboxyl (COOH)-terminal region.

Ligand-independent transcription activation mostly influences amine (NH2)-terminal region of PPAR-α structure. Basicly, like other nuclear receptor, PPAR-α consists of three specific regions, namely NH2-terminal region, DNA-binding region (bind to DNA) and COOH-terminal region (bind to ligand, retinoid X-receptor or RXR, and coactivator or corepressor). PPAR-α could be activated through phosphorylation of amino acid (mostly serine 12, 21 or 73) by some types of

kinase enzymes such as mitogen activating protein kinase (MAPK) and protein kinase A (PKA) [1, 3, 10]. The effect of purple sweet potato extract (*Ipomoea batatas)* (which predominantly contained anthocyanin), on PPAR expression had been reported in study conducted by Mi *et al*. (2018).However this study evaluated the PPAR-γ expression, not the PPAR-α expression.

As we know that PPAR-γ is mostly expressed in white adipose tissues, not in the liver. In this study, it was revealed that induction of high fat diet together with carbon tetrachloride injection on rats for ten weeks resulted in significant enhanced of TNF-α, IL-1ß, NFκB and HMGB-1 expression, as well as significant decline of IL-4, IL-13 and PPAR-γ expression. Intervention with 240 mg/kgBW of purple sweet potato extract potentially led to significant decreased expression of TNF-α, IL-1ß, NFκB and HMGB-1 mRNA, as well as significant increased expression of IL-4, IL-13 and PPAR-γ mRNA, compare to control group [6].

Our study showed that intervention with purple sweet potato extract (anthocyanin-rich plants) resulted in enhanced PPAR-α expression in hepatotoxic rats. Similar findings had demonstrated by Farrell *et al.* (2015) and Sozanski *et al.* (2016). However, intervention in research done by Farrel *et al.* (2015) was slightly different from our study.

Study by Farrell *et al.* (2015) evaluate black elderberry extract which was given for 16 weeks for liver protection through increasing PPAR-α expression. Black elderberry extract also contained anthocyanin.[13] Research performed by Sozanski *et al*. (2016) had also stated that anthocyanin-rich plants contributed to increase PPAR-α expression. However, the intervention used in this study was *Cornus mas* fruit *(*cornelian cherry*)* extract [5].

Other class of flavonoid which had also proved to influence PPAR-α was isoflavone. Previous evidence had reported that isoflavone could induce PPAR-α expression in the liver on fatty liver disease. Isoflavone had been proved as PPAR-α agonist which would promote PPAR-α expression and subsequently suppressed oxidative stress and inflammation. Enhanced PPAR-α expression would lead to increase of antiinflammatory and antioxidant protein expression (SOD, CAT, GPX, thioredoxin, heme oxygenase) [14-15].Some evidences had also suggested the effect of anthocyanin-rich plants to increase PPAR-α expression for liver protection in rats induced with high fat diet, namely research by Yan *et al* (2017).

Jang *et al*. (2012) and Felix *et al*. (2017). Study performed by Yan *et al*. (2017) demonstrated that mulberry extract potentially increased expression of PPAR-α, as well as AMPK and MAPK [16]. Black rice extract had also proved to increase PPAR-α expression and activation on rats induced by high fat diet [17]. Research conducted by Felix et al. (2017) represented that black rice and brown rice extract could enhance PPAR-α expression on diabetic and obese rats [18].Anthocyanin effect on PPAR-α expression might be presumably associated with its role as agonist of PPAR in the liver. However its effect on PPAR had been mostly related to lipid metabolism in the liver [19].

Other flavonoids such as isoflavones had also shown similar evidence on PPAR expression (PPAR-α or PPAR-γ) in the liver.[20] Polyphenol, in general, also had similar effect on PPAR-γ and PPAR-α expression in the liver [21].Purple sweet potato extract had been proved to posses antioxidant and antiinflammatory effect in many studies. However, its antioxidant activity was mostly related to activation of Nrf2, a transcription factor which promote the expression of many antioxidant proteins (GSTA2, HO-1, NQO1, GCS, GCL).

Its antiinflammatory activity was mostly related to inhibition of NFκB, a transccription factor responsible for inducing the expression of some proinflammatory proteins, including inflammatory enzymes, receptors, cytokines, chemokines, adhesion molecules, etc [22-25]. Our results had supported that purple sweet potato extract could enhance PPAR-α expression and activation.

This effect might presumably contribute to its antioxidant and antiinflammatory activity. PPAR-α expression could be increased by stimulation of other transcription factors such as growth factor and Nrf2. Nrf2 and PPAR-α might affect upregulation of each other [26]. In our study, the higher expression of PPAR-α in the liver in intervention group compare with that in control group might also be associated with PPAR-α activation. When a ligand bind to

PPAR-α, it would prevent proteasomic degradation of PPAR-α. Contrary, when no ligand interacts to it, it will be rapidly degraded by proteasome through ubiquitination process in only few hours [1, 26].

Conclusions

In conclusion, purple sweet potato ethanolic extract dose 200 mg/200 grams rat/day potentially showed hepatoprotective effect in Wistar rats induced by isoniazid and rifampicin. Purple sweet potato ethanolic extract dose 200 mg/200 grams rat/day could increase PPAR-α expression and activation in the liver.

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References

1. Neels JG and Grimaldi PA (2014) Physiological functions of peroxisome proliferator-activated receptor ß. Physiology Review, 94: 795-858.
2. Nan Y-M, Wang R-Q, Fu N (2014) Peroxisome proliferator activated receptor α, a potential therapeutic target for alcoholic liver disease. World Journal of Gastroenterology, 20(25): 8055-8060.
3. Grygiel-Gorniak B (2014) Peroxisome proliferator-activated receptors and their ligands: nutritional and clinical implications: a review. Nutrition Journal, 13(17): 1-10.
4. Hassan HM, Guo H, Yousef BA, Guerram M, Hamdi AM, Zhang L, Jiang Z (2016) Role of inflammatory and oxidative stress, cytochrome p450 2e1 and bile acid disturbance in rat liver injury induced by isoniazid and lipopolysaccharide cotreatment. Antimicrobial Agents and Chemotherapy, 60(9): 5285-5293.
5. Sozanski T, Kucharska AZ, Rapak A, Szummy D, Trocha M, Merwid-Lad A, et al (2016) Iridoid-loganic acid versus anthocyanins from the Cornus mas fruits (corneelian cherry): common and different effects on diet-induced atherosclerosis, PPARs expression and inflammation. Atherosclerosis, 254: 151-160.
6. Mi W, Han F, Liang J, Liang Y, Guan B, Xu H (2018) Purple sweet potato anthocyanins attenuates steatohepatitis induced by high fat diet combined with carbon tetrachloride in rats. Wei Sheng Yan Jiu, 47(4): 517-524.
7. Miguel MG (2011) Anthocyanins: antioxidant and or antiinflammatory activities. Journal of Applied Pharmaceutical Science, 1(6): 7-15.
8. Artini IGA, Jawi IM, Astawa INM, Sutirta-Yasa IWP (2020) Developing animal model for analysing liver toxicity of isoniazid and rifampicin combination. Journal of Global Pharma Technology, 12(1): 391-396.
9. Pawlak M, Lefebvre P, Staels B (2015) Molecular mechanism of PPARα action and its impact on lipid metabolism, inflammation and fibrosis in non-alcoholic fatty liver disease. Journal of Hepatology, 62: 720-733.
10. Brunmeir R and Xu F (2018) Functional regulation of PPARs through post-translational modifications. International Journal of Molecular Sciences, 19(1738): 1-16.
11. Iwamoto F, Umemoto T, Motojima K, Fujiki Y (2011) Nuclear transport of peroxisome proliferator activated receptor α. The Journal of Biochemistry, 149(3): 311-319.
12. Esposito E, Cuzzocrea S, Meli R (2006) Peroxisome proliferator-activated receptors and shock state. The Scientific World Journal, 6: 1770-1782.
13. Farrell NJ, Norris GH, Ryan J, Porter CM, Jiang C, Blesso CN (2015) Black elderberry etract attenuates inflammation and metabolic dysfunction in diet-induced obese mice. British Journal of Nutrition, 114: 1123-1131.
14. Kim T and Yang Q (2013) Peroxisome proliferator-activated receptors regulate redox signaling in the cardiovascular system. World Journal of Cardiology, 5(6): 164-174.

15. Qiu L-X and Chen T (2015) Novel insights into the mechanisms whereby isoflavones protect against fatty liver disease. World Journal of Gastroenterology, 21(4): 1099-1107.

16. Yan F, Chen Y, Azat R, Zheng X (2017) Mulberry anthocyanin extract ameliorates oxidative damage in hepG2 cells and prolongs the lifespan of caenorhabditis elegans through mapk and nrf2 pathways. Oxidative Medicine and Cellular Longevity, 2017: 1-12.

17. Jang H, Park M, Kim H, Lee Y, Hwang K, Park J, et al (2012) Black rice (oryza sativa l.) extract attenuates hepatic steatosis in c57bl/6j mice fed a high-fat diet via fatty acid oxidation. Nutrition and Metabolism, 9(27): 1-11.

18. Felix ADR, Takahashi N, Takahashi M, Katsumata-Tsuboi R, Satoh R, Hui TS, et al (2017) Extract of black and brown rice powders improve hepatic lipid accumulation via the activation of pparα inobese and diabetic model mice. Bioscience, Biotechnology, and Biochemistry, 81(11): 2209-2211.

19. Eslamparast T, Eghtesad S, Poustchi H, Hekmatdoost A (2015). Recent advances in dietary supplementation, in treating non-alcoholic fatty liver disease. World Journal of Hepatology, 7(2): 204-212.

20. Medjakovic S, Mueller M, Jungbauer A (2010) Potential health-modulating effects of isoflavones and metabolites via activation of ppar and ahr. Nutrients, 2: 241-279.

21. Dominguez-Avila JA, Gonzalez-Aguilar GA, Alvarez-Parrilla E, de la Rosa LA (2016) Modulation of ppar expression and activity in response to polyphenolic compounds in high fat diets. International Journal of Molecular Sciences, 17: 1002-1018.

22. Pall ML and Levine S (2015) Nrf2, a master regulator of detoxification and also antioxidant, anti-inflammatory and other cytoprotective mechanisms, is raised by health promoting factors. Acta Physiologica Sinica, 67(1): 1-18.

23. Lee Y, Yoon Y, Yoon H, Park H, Song S, Yeum K (2017) Dietary anthocyanins against obesity and inflammation. Nutrients, 9: 1089-1104.

24. Yang L, Xian D, Xiong X, Lai R, Song J, Zhong J (2018) Proanthocyanidins against oxidative stress: from molecular mechanisms to clinical applications. Biomed Research International, 2018: 1-11.

25. Vendrame S and Klimis-Zacas D (2015) Anti-inflammatory effect of anthocyanins via modulation of nuclear factor-κB and mitogen-activated protein kinase signaling cascades. Nutrition Reviews, 73(6): 348-358.

26. Korbecki J, Bobinski R, Dutka M (2019) Self-regulation of the inflammatory response by peroxisome proliferator-activated receptors. Inflammation Research, 68(6): 443-458.

