Grape seed proanthocyanidin extract and anti-platelet drug (clopidogrel) alleviates non-alcoholic fatty liver disease via inhibition of hepatic inflammation in rats

Samy A. Hussein1, Omayma A. R. Abo zaid1, Mohamed A. Azab2, Soheir K. Mohamed1,*

1 Biochemistry Department, Faculty of Veterinary Medicine, Benha University, Egypt.
2 Physiology Department, Faculty of Veterinary Medicine, Benha University, Egypt.

ABSTRACT

The initiation and progression of nonalcoholic fatty liver disease (NAFLD) include imbalanced lipid metabolism, insulin resistance (IR) and oxidative stress. Consequently, grape seed proanthocyanidin extract (GSPE) showed very beneficial effect against the NAFLD metabolic disruptions, via its antioxidant, anti-inflammatory and lipolytic properties. Clopidogrel also can reduce the oxidative stress and inflammation. Thirty-two male albino rats were divided into four equal groups of 8 rats; Group I: Normal control group (G1): Rats fed ordinary normal diet for 12 weeks. Group II: NAFLD group (G2): Rats fed HFD-diet for 6 weeks for NAFLD induction, followed by administration of GSPE or in combination with Clopidogrel (G4) for another 6 weeks. The results showed that treatment with GSPE (G3) or GSPE and Clopidogrel (G4) significantly decreased the higher serum total cholesterol, triacylglycerols and ferritin concentrations that were observed in NAFLD induced group (G2). Interestingly, the hepatic inflammatory mediators VEGF, PDGF α and MAPK gene expression levels were significantly down-regulated in GSPE (G3) or GSPE and Clopidogrel co-treated (G4) groups as compared with NAFLD-induced group (G2). In conclusion, treatment with GSPE alone or with Clopidogrel is a potent hypolipidemic and anti-inflammatory, restoring hepatic cell function and alleviates the oxidative stress and inflammation related to NAFLD in rats.

1. INTRODUCTION

Oxidative stress, lipotoxicity, and inflammation play key role in the progression of NAFLD (Yang et al., 2019). When IR occurs, liver becomes more vulnerable to hyperinsulinenia-induced ‘multi-hit’ events involving the release of reactive oxygen species (ROS), pro-fibrogenic factors and pro-inflammatory mediators from impaired organelles (Xiao et al., 2013). Grape seeds extract (GSE) improved the antioxidant defense, and prevented the HFD-induced inflammation, through the reduction of pro-inflammatory cytokines (da Costa et al., 2017), while Clopidogrel is the most widely prescribed anti-platelet for the amelioration of oxidative stress, and endothelial dysfunction (Ramadan et al., 2014). Accordingly, the present study was designed to investigate the possible protective and therapeutic efficacy of GSPE or in combination with Clopidogrel by evaluation of serum lipids profile, ferritin in addition to the molecular analysis of some inflammatory mediators gene expression levels in liver tissues as VEGF, PDGFα, and MAPK in the experimental model of NAFLD in rats.

2. MATERIAL AND METHODS

2.1. Experimental animals

Thirty-two albino male rats, 4-5 week’s old with ~150–200g weight were used in this experiment. All rats were acclimatized for two weeks before the experiment, and were fed regularly. The study protocol was approved by the Animal Care and Use commission of Benha University in compliance with the National Institute of Health Guider for the Care and Use of research Animals.

2.2. The antioxidant agent and drugs

2.2.1. Grape seed proanthocyanidin extract

The antioxidant agent GSPE was purchased from Al Debeiky Pharma Company for Pharmaceutical industries, Al Obour, Cairo, Egypt. Preparation: GSPE was freshly dissolved in 100% Dimethyl sulfoxide (DMSO) and diluted to the appropriate concentration by saline and administered orally in a dose of (200 mg/kg/day) (Da Costa et al., 2017).

2.2.2. Antiplatelet drug

The pharmacological anti-platelet drug (Plavix®) 75 mg film coated tablets with the active ingredient Clopidogrel
hydrogen sulfate, was obtained from Sanofi pharma Bristol-Myers Squibb SNC Paris-France. 

**Preparation:** Clopidogrel was dissolved in saline and administered orally by intra-gastric tube at a dose of 6.75 mg/kg b.w./day (Ahn et al., 2019).

### 2.3. Experimental design:

Rats were randomly divided into four equal groups (8each) as follows:

**Group I:** Normal control group (G1): Rats received no drugs and was provided with ordinary normal diet for 12 weeks, and it was served as a control group.

**Group II:** NAFLD group (G2): Rats provided with HFD-diet for 6 weeks for the induction of NAFLD (Li et al., 2014), followed by ordinary normal diet feeding for another 6 weeks.

**Group III:** NAFLD+GSPE treated group (G3): Rats fed HFD for 6 weeks (NAFLD) followed by administration of GSPE for another 6 weeks.

**Group IV:** NAFLD + GSPE + clopidogrel treated group (G4): Rats fed HFD for 6 weeks (NAFLD), followed by GSPE for another 6 weeks and with clopidogrel (6.75 mg/kg b.w./day) for additional 6 weeks.

### 2.4. Sampling

**2.4.1. Blood samples:**

All rats were sacrificed, after 12 week of the experiment onset by cervical decapitation according to Animal Ethics Committees, and blood samples for serum separation were collected, then centrifuge for 15 minutes at 3000 rpm and the separated serum was kept in Eppendorf tubes and stored at 20 °C in deep freezer for determination of the of total cholesterol, triacylglycerols and ferritin concentrations according to the method described by NCEP expert panel. (1988), Stein, (1987), and Dawson et al. (1992), respectively.

**2.4.2. Tissue samples:**

Liver of all rats were removed, cleaned by rinsing with cold saline, immediately kept in liquid nitrogen and stored at -80°C till RNA extraction for the real time quantitative polymerase chain reaction (qPCR) analysis of the hepatic gene expression levels of vascular endothelial growth factor (VEGF), platelet-derived growth factor-α (PDGFα) and mitogen activated protein kinase (MAPK).

**2.4.3. Molecular analysis**

The mRNA expressions content of VEGF, PDGFα and MAPK in the liver tissues of the rats were determined using qPCR. Samples were processed simultaneously (Bush et al., 2001), B-actin was used as the load control. Total RNA was isolated using High Kit for pure RNA isolation (Thermo Scientific, Fermentas, #K0731), the produced cDNAs from the reverse transcribed template RNAs using Revert Aid™ H Minus Reverse transcriptase kit (#E90451, Thermo Scientific, Fermentas, USA) were amplified on Fast start Universal SYBR Green Master (Roche, GER). The target gene was normalized with β-actin by the 2ÅΔCt method (Livak and Schmittgen, 2001).

| Gene | Forward primer (5 ' — 3 ') | Reverse primer (5 ' — 3 ') |
|------|----------------------------|---------------------------|
| PDCGFα | CCAACCTGGAACCCAGACCAT | AGGAGTGACGAAAAGCAGAT |
| VEGF | TACATGGGTCAGCAAACCTCAC | CCTGCAGACACTGTCTC |
| MAPK | AGGCGCAGTTGACCTTTT | CTTGCGAGGTAGAAGTTG |
| β-actin | AGTCCTCCACCTCCCTCCAAAG | AGGAATGCTGCCATCCTCC |

### 2.5. Statistical analysis

Results were expressed as mean ± SE using SPSS (18.0 software, 2011). Using one-way ANOVA followed by Duncan's test data were analysed. Values were statistically significant at P≤0.05.

### 3. RESULTS

This study data in table (2) illustrate that, serum total cholesterol, triacylglycerols and Ferritin levels were significantly increased in the NAFLD group (G2), when compared with the control group (G1), while were significantly decreased in GSPE (G3), and GSPE + clopidogrel (G4) treated groups, comparing with the NAFLD rats (G2). Likewise, the obtained qPCR results in table (3) showed significant up-regulation (P≤0.05) in VEGF, PDGFα and MAPK gene expression levels in the NAFLD rats (G2), when compared to the controls (G1), while were significantly downregulated in the GSPE (G3) and GSPE + clopidogrel (G4) treated groups, comparing with the NAFLD group (G2). However, no significant difference was noticed between (G3) and (G4) and their expression levels remained significantly higher than the controls (G1).

### 4. DISCUSSION

Currently, a beneficial treatment of NAFLD may be through the targeting of NAFLD by treating obesity and treating NAFLD by targeting fat redistribution (Niccolai et al., 2019). The obtained data in table (2) revealed significant increase in serum total cholesterol and triacylglycerols concentrations in HFD-induced NAFLD rats (G2), in comparison with the control rats (G1). Likewise, Oriquat et al. (2018) showed that, hepatic tissues of NAFLD rats showed significantly higher levels of triacylglycerols (~225%) and total cholesterol when compared with control
rats. Actually, serum lipid profile correlates significantly with NAFLD severity (Françque et al., 2016), as the obese state in the liver promotes lipogenesis and elicits mitochondrial dysfunction resulting in hepatic fatty acids and lipid overload (Leonetti et al., 2018). Interestingly, treatment of the NAFLD rats with GSPE (G3) showed effective improvement in the serum triacylglycerols and total cholesterol levels illustrated by their significant decrease, comparing with the NAFLD untreated rats group (G2). Similarly, GSPE (25 or 200 mg/kg/b.wt. /day) decreased the plasma triacylglycerols level by 28% or 25%, respectively in obese rats (Pascual-Serrano et al., 2018). GSPE treatment reduced adiposity, plasma triacylglycerols, and oxidative stress in the liver (Leonetti et al., 2018), in which the most recognized mechanism of action include inhibition of LPO and avoiding the associated ROS production (Rodriguez-Pérez et al., 2019).

Moreover, administration of Clopidogrel with GSPE co-treatment to the NAFLD-induced rats (G3), showed significant decrease in the serum levels of triacylglycerols and total cholesterol, compared to the NAFLD untreated rats (G2). The obtained data agree with Hadi et al. (2013), who observed non-significant decrease of serum triacylglycerols and total cholesterol concentrations after Clopidogrel treatment in comparison with atherogenic control rabbits fed on HFD. Referring that antithrombotic treatment slows down progression of acute or chronic liver failure in animal models (Fujita et al., 2008), the clinical benefit of Clopidogrel may be attributed to improvement of oxidative stress and reducing of inflammation (Ramadan et al., 2014).

The obtained data shown a significant increase in serum ferritin concentration in the NAFLD-induced rats (G2) as compared to control (G1). Similarly, Ryan et al. (2018) stated that, serum ferritin was higher in NAFLD patients compared to controls. Ferritin plays a pro-inflammatory role during the progression of the liver disease, in which its elevated serum level is related to IR and hepatocyte damage (Du et al., 2017).

Furthermore, a clear protective role was observed following administration of GSPE to NAFLD induced rats (G3), compared to the NAFLD untreated group (G2), clarified by the significant decrease in serum ferritin concentration. Likewise, Lafay et al. (2009) showed that, after supplementation of grape extract (400 mg) ferritin were significantly decreased in male rats. Regarding that high iron stores is reflected by increased circulating ferritin (Basuli et al., 2014), the antioxidant property of GSPE is closely related to its iron-chelating effects (Bagchi et al., 2014), and/or its ability to directly scavenge free radicals, or indirectly modulating pro-oxidant enzymes (Fraga and Oteiza, 2011).

Additionally, GSPE and clopidogrel-co-treated NAFLD induced rats (G4) also showed significant improvement of ferritin concentrations, comparing with the NAFLD untreated rats (G2). Clopidogrel can ameliorate the endothelial dysfunction (Ramadan et al., 2014), caused by release of ROS (Ostad et al., 2011), in which toxic effect of increased iron resulted in altering of endothelial function and decreasing of vascular reactivity (Sullivan, 2005).

The obtained qPCR results in table (3) elucidate significant upregulation of the hepatic expression levels of the inflammatory mediators VEGF, PDGFα and MAPK in the NAFLD-induced rats (G2), in comparison with the control (G1). Similarly, Miura et al. (2019) observed that, fat overload by HFD increase liver Kupffer cells and macrophages VEGF expression. Moreover, when liver damage occurs, PDGF may be highly-expressed in activated hepatic stellate cells (HSC), injured endothelial cells and macrophages (Ying et al., 2017). Furthermore, Yan et al. (2017) had stated that, obesity promotes NAFLD, in a rat model of NAFLD, by over-expression of c-Jun NH2-terminal kinase (JNK)-1. VEGF may be used as an independent predictor of NAFLD (Wu et al., 2015), as it is a key factor in angiogenesis and tissue remodeling, and has a role in IR and inflammation, that are important characteristics in the development of NAFLD (Hong et al., 2019). Additionally, VEGF and PDGF are produced and released by several liver cells during chronic liver diseases progression (Novo et al., 2014), in which binding of PDGF to their receptors-α and -β activates predominantly the Ras-MAPK pathway leading to the cellular reactions and molecular changes (Ying et al., 2017). Actually, the MAPK signaling pathway plays a key role in the NAFLD development (Ye et al., 2019), and MAPKs participate in the hepatic metabolism process, notably, the hepatic MAPKs are activated by stress responses, as p38 MAPKs and JNKs can be activated by ROS (Lawan and Bennett, 2017).

Interestingly, treatment of NAFLD-induced rats with GSPE resulted in significant down-regulation of the hepatic expression levels of VEGF, PDGFα and MAPK (G3), compared to the NAFLD untreated rats (G2). Likewise, Huang et al. (2012) had reported that GSPE inhibit VEGF as one of multiple modulating signaling pathways to exhibit anti-angiogenic effects against cancer. Also healing repair effects by GSPE, via VEGF expression blocking can be a possible mechanism that can be mediated through inhibition of protein kinase (Akt) activation (Lu et al., 2009), and the inhibitory effect of PDGFR signaling by procyanidin-B2 may be via inhibition of ligand binding to the receptor and/or inhibition of the receptor's intrinsic tyrosine kinase activity (Rosenkranz et al., 2002), namely α- and β-PDGFR receptors (Rosenkranz and Kazlauskas, 1999). Additionally, GSPE pre-treatment suppressed the activation of Akt and MAPKs (JNK, Extracellular signal-regulated kinase (ERK) and p38) (Lee et al., 2017). In fact, GSPE may be a novel treatment of hepatic inflammation, exerting its anti-inflammatory effects by the attenuation of MAPK, via suppression of the JNK, and nuclear factor-kappaB (NF-κB) signaling pathways, in which MAPK/ERK-nuclear factor erythroid 2-related factor (Nrf2) is crucial to proanthocyanidin-mediated antioxidation and anti-inflammation (Nie and Stürzenbaum, 2019).

Furthermore, GSPE and clopidogrel co-treatment to the NAFLD-induced rats (G4) showed significant down-regulation of VEGF, PDGFα and MAPK hepatic expression levels, comparing with the NAFLD untreated rats (G2). Similarly, Coimbra et al. (2014) clarified that, Clopidogrel enhanced angiogenesis by reducing PDGF expression during periodontal repair, thereby, reducing the inflammation. Moreover, Clopidogrel can directly reduce the synthesis of VEGF (Kou et al., 2018). Interestingly, transforming growth factor (TGFβ) pathway can mediate the activation of the JNK and p38/MAPK pathways (Papageorgis and Stylianos, 2015), and the inhibition of MAPK cascade leads to reduction of JNK activity, which may improving IR (Prattali et al., 2005), thereby the down-regulation of TGFβ by Clopidogrel treatment (Joshi et al., 2016) in NAFLD may explain its positive effect. Additionally, Clopidogrel exerts beneficial vascular effects via the marked oxidative stress, reduction and abolishing of the vascular inflammatory responses and remodeling (An et al., 2018).
It could be concluded that, treatment with GSPE or with antiplatelet drug alleviates the oxidative hepatic stress and inflammation associated with NAFLD clarified by improving hyper-lipidemia and reduction of the inflammatory mediators. In fact, GSPE may be a novel treatment of hepatic inflammation in fatty liver disease, exerting its anti-inflammatory effect by the attenuation of MAPK, through suppression of the VEGF and PDGFr signaling pathways.

6. REFERENCES

1. Ahn, K.T., Seong, S-W., Choi, U.L., Jin, S-A., Kim, J.H., Lee, J-H., Choi, S-W., Jeong, M.H., Choe, S.C., Kim, Y.J., Kim, C.J., Kim, H-S., Cho, M-C., Gwon, H-C., and Jeong, J-O. 2019. Comparison of 1-year clinical outcomes between prasugrel and ticagrelor versus clopidogrel in type 2 diabetes patients with acute myocardial infarction underwent successful percutaneous coronary intervention. Medicine, 98: 11(e14833).

2. An, X., Jiang, G., Cheng, C., Lv, Z., Liu, Y., and Wang, F. 2018. Inhibition of Platelets by Clopidogrel Suppressed Ang II-Induced Vascular Inflammation, Oxidative Stress, and Remodeling. J Am Heart Assoc, 7(21): e009600.

3. Bagchi, D., Swaroop, A., Preuss, H.G., and Bagchi, M. 2014. Free radical scavenging, antioxidant and cancer chemoprevention by grape seed proanthocyanidin: An overview. MetaRes Fundam Mol Mech Mutagen, 768: 69-73.

4. Basuli, D., Stevens, R.G., Torti, F.M., and Torti, S.V. 2014. Epidemiological associations between iron and cardiovascular disease and diabetes. Front Pharmacol, 5: 117.

5. Combra, J.S., Steffens, J.P., Jr, C.R., Graves, D.T., and Spolidorio, I.C. 2014. Clopidogrel enhances periodontal repair in rats through decreased inflammation. J Clin Periodontol, 41(3): 295-302.

6. da Costa, G.F., Santos, I.B., de Bem, G., Cordeiro, V.S.C., da Costa, C.A., Carvalho, LCRM, Ognibene, D.T., Resende, A.C., and de Moura, R.S. 2017. The beneficial effect of anthocyanidin-rich vitis vinifera L. grape skin extract on metabolic changes induced by high-fat diet in mice involves antiinflammatory and antioxidant actions. Phytother Res, 31: 1621-1632.

7. Dawson, D.W., Fish, D.L., and Shackleton, P. 1992. The accuracy and clinical interpretation of serum ferritin assays. Clin Lab Haematol, 14(1): 47-52.

8. Du X., She, E., Gelbart, T., Truksa, J., Lee, P., Xia, Y., Khovananth, K., Mudd, S., Mann, N., MoreSCO, E.M.Y., Beutler, E., and Beutler, B. 2008. The serine protease TMPRSS6 is required to sense iron deficiency. Science, 320: 1088-1092.

9. Fraga, C.G. and Oreiza, P.I. 2011. Dietary flavonoids: Role of (−)-epicatechin and related proanthocyanins in cell signaling. Free Radic Biol Me, 51: 813-823.

10. Francque, S.M., van Graaft, D., and Kwanten, W.J. 2016. Non-alcoholic fatty liver disease and cardiovascular risk: Pathophysiological mechanisms and implications. J Hepatol, 65(2): 425-443.

11. Fujita, K., Nozaki, Y., Wada, K., Yonedo, M., Endo, H., Takahashi H., Iwasaki, T., Inamori, M., Abe Y., Kobayashi, N., Kirikoshi, H., Kubota, K., Saito, S., Nagashima, Y., and Nakajima, A. 2008. Effectiveness of antiplatelet drugs against experimental non-alcoholic fatty liver disease. Gut, 57: 1583-1591.

12. Hadi, N.R., Mohammad, B.I., Ajeena, I.M., and Sahib, H.H. 2013. Antithrombotic Potential of Clopidogrel: Antioxidant and Anti-Inflammatory Approaches. Biomed Res Int, https://doi.org/10.1155/2013/790263.

13. Hong, W., Songsong, L., Liyan, W., Beihui, H., Jiang, J., and Zhiyun, L. 2019. Prediction of VEGF-C as a key target of pure total flavonoids from citrus against NAFLD in mice via network pharmacology. Frontiers in Pharmacology, 04: 92(3): 130-7.

14. Huang, S., Yang N., Liu, Y., Hu, L., Zhao, J., Gao, J., Li, Y., Li, C., Zhang, X., and Huang, T. 2012. Grape seed proanthocyanidins inhibit angiogenesis via the downregulation of both vascular endothelial growth factor and angiopoietin signaling. Nutrition Research, 32(7): 530-536.

15. Joshi, N., Kopeck, A.K., Ray, J.L., and Luyendyk, J.P. 2016. Inhibition of PAR-4 and P2Y12 receptor-mediated platelet activation produces distinct hepatic pathologies in experimental xenobiotic-induced cholestatic liver disease. Toxicol, 365: 9-16.

16. Koi, N., Xue, M., Wang, L., Zang, M.X., Qu, H., Wang, M.M., Miao, Y., Yang, B., and Shi, D.Z. 2018. Panax quinquefolius saponins combined with dual antiplatelet drug therapy alleviate gastric mucosal injury and thrombogenesis through the COX/PG pathway in a rat model of acute myocardial infarction. PLoS One, 13(3): e0194082.

17. Lafay, S., Jan, C., Nardon, K., Lemaire, B., Ibarra, A., Rollier, M., Houvenaeghel, M., Juwel, C., and Cara, L. 2009. Grape extract improves antioxidant status and physical performance in elite male athletes. J Sports Sci Med, 8(3): 468-480.

18. Lawan, A and Bennett, A.M. 2017. Mitogen-Activated Protein Kinase Regulation in Hepatic Metabolism. Trends Endocrinol Metab, 28(12): 868-878.

19. Lee, J.W., Kim, Y.L., Kim, Y., Choi, M., Min, S., Joo, Y.H., Yim, S.V., and Chung, N. 2017. Grape seed proanthocyanidin inhibits inflammatory responses in hepatic stellate cells by modulating the MAPK, AKT and NF-κB signaling pathways. Int J Mol Med, 40(1): 226-234.

20. Leonetti, D., Soleti, R., Cler, N., Vergori, L., Jacques, C., Duluc, L., Dourguia, C., Martinez, M.C., and Andriantistihaina, R. 2018. Extract enriched in flavan-3-ols and mainly proanthocyanin dimers improves metabolic alterations in a mouse model of obesity-related disorders partially via estrogen receptor alpha. Front Pharmacol, 9: 406. doi:10.3389/fphar.2018.00406

21. Li, S., Meng, F., Liao, X., Wang, Y., Sun, Z., Guo, F., Li, X., Meng, M., Li, Y., and Sun, C. 2014. Therapeutic role of ursolic acid on ameliorating hepatic steatosis and improving metabolic disorders in high-fat diet-induced non-alcoholic fatty liver disease in rats. PLoS ONE, 9(1): e86724.

22. Liu, X., Qu, J., Zhao, S., You, B., Ji, X., Wang, Y., Cui, X., Wang, Q., and Gao, H. 2012. Grape seed proanthocyanidin extract alleviates ouabain-induced vascular remodeling through evaluation of regenerative function. Mol Med Rep, 6(5): 949-54.

23. Livak, K.J. and Schmittgen, T.D. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2−ΔΔCT Methods, 25(4): 402-408.

24. Miura, K., Ohnishi, H., Morimoto, N., Minami, S., Ishoka, M., Watanabe, S., Tsukui, M., Takakoa, Y., Nomoto, H., Isoda, N., and Yamamoto, H. 2019. Ezetimibe suppresses development of liver tumors by inhibiting angiogenesis in mice fed a high-fat diet. Cancer Sci, 110(2): 771-783.

25. NCEP expert panel, 1988.(NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. Evaluation, and Treatment of High Blood Cholesterol in Adults. Circulation, 148: 36-69.

26. Niccolai, E., Boem, F., Russo, E., and Amedei, A. 2019. The gut-brain axis in the neuropsychological disease model of obesity: A classical movie revised by the emerging director “Microbiome”. Nutrients, 11: 156.

27. Niederreiter, L. and Tög, H. 2018. Cytokines and fatty liver diseases. Liver Res, 2: 140.

28. Novo, E., Cannito, S., and Paternostro, C. 2014. Cellular and molecular mechanisms in liver fibrogenesis. Arch Biochem Biophys, 548: 20-37.

29. Oriqat, G.A. 2018. Therapeutic effects of Spirulina against experimentally-induced nonalcoholic fatty liver in rats may involve miR-21, -34a and -122. Meta Genome, 18: 115-121.

30. Ostad, M.A., Nick, E., Paixao-Gatinho, V., Schnorbus, B., Schiewe, K., Tschentscher, P., Munzel, T., and Warnholtz, A. 2011. Lack of evidence for pleiotropic effects of clopidogrel on endothelial function and inflammation in patients with stable coronary artery disease: results of the double-blind, randomized CASSANDRA study. Clin Res Cardiol, 100: 2-36.

31. Papageorgis, P. and Stylianopoulos, T. 2015. Role of TGFβ in regulation of the tumor microenvironment and drug delivery (review). Int J Oncol, 46: 933-43.

32. Pascual-Serrano, A., Bladé, C., Suárez, M., and Arola-Arna, A. 2018. Grape Seed Proanthocyanidins Improve White Adipose Tissue Expansion during Diet-Induced Obesity

112
