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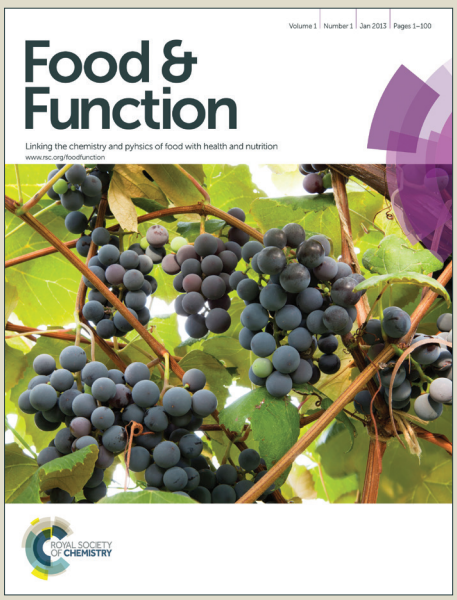

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A novel PPARgamma agonist monascin potentially applied in diabetes prevention

Running title: Anti-diabetic effect of monascin

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Abstract

Edible fungi of the *Monascus* species have been used as traditional Chinese medicine in eastern Asia for several centuries. *Monascus*-fermented products possess a number of functional secondary metabolites, including the anti-inflammatory pigments monascin and ankaflavin. Monascin has been shown to prevent or ameliorate several conditions, including hypercholesterolemia, hyperlipidemia, diabetes, and obesity. Recently, monascin has been shown to improve hyperglycemia, attenuate oxidative stress, inhibit insulin resistance, and suppress inflammatory cytokines production. In our recent study, we have found that monascin is a peroxisome proliferator-activated receptor-gamma (PPARgamma) agonist. This PPARgamma agonist activity had been investigated and exerted benefits for inhibition of inflammation in methylglyoxal (MG)-treated rats, prevention of pancreas impairment caused advanced glycation endproducts (AGEs), promotion of insulin expression in vivo and in vitro, and attenuated carboxymethyllysine (CML)-induced hepatic stella cells (HSCs) activation in past several years. Moreover, our studies also demonstrated that monascin also activated nuclear factor-erythroid 2-related factor 2 (Nrf2) in pancreatic RIN-m5F cell line thereby invading methylglyoxal-resulted in pancreas dysfunction. In this review, we focus on the chemo-preventive properties of monascin against metabolic syndrome through PPARgamma and Nrf2 pathways.

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Keywords: monascin, peroxisome proliferator-activated receptor-gamma (PPARgamma) agonist, methylglyoxal (MG), advanced glycation endproducts (AGEs), nuclear factor-erythroid 2-related factor 2 (Nrf2)

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1. Introduction

Monascus was classified and named in 1884 by the French scientist van Tieghem.[1] The genus *Monascus* belongs to the family Monascaceae, order Eurotiales, class Ascomycetes, phylum Ascomycota, and kingdom Fungi. Thus far, 58 *Monascus* strains have been deposited in the American Type Culture Collection; however, most strains belong to only 3 species: *Monascus pilosus*, *Monascus purpureus*, and *Monascus ruber*.[2] *Monascus*-fermented products, especially those produced by solid-state rice fermentation, have been used as food colorants and dietary material for more than 1,000 years. *Monascus*-fermented rice, also known as red mold rice, is a common foodstuff and traditional health remedy in Asian countries. Red mold rice, largely produced by *M. purpureus* contains various chemical components, some of which have been purified and identified, including monascolins,[3,4] γ-aminobutyric acid,[5] pigments such as monascin and ankaflavin,[6] and antioxidant such as dimerumic acid.[7] It was reported that monascin is the major constituent of the azaphilonoid compound. The structure of monascin is shown in **Fig. 1a**, and which has been recently reported to be a PPARgamma agonist in our study **(Fig. 1b)**.[8] It is suggests that monascin plays a role for PPARgamma activation.

Hyperglycemia is associated with protein glycation; advanced glycation end products (AGEs) are generated by the nonenzymatic interaction between

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carbohydrates and proteins. AGEs have properties to generate free radicals and undergo autoxidation to generate other reactive intermediates, thereby resulting in the development of diabetes.[9] Methylglyoxal (MG) is a highly reactive dicarbonyl metabolite produced during glucose metabolism[10] and is a major precursor of AGEs involved in the pathogenesis of diabetes and inflammation. Studies suggest that AGEs and MG can generate large amounts of proinflammatory cytokines through receptor for AGEs (RAGE) activation, and these results are related to the modulation of inflammatory molecules through oxidative stress.[10]

PPARgamma ligands are reported to activate the phosphatidylinositol 3-kinase/Akt pathway, which can elevate insulin sensitivity to downregulate blood glucose.[11] Moreover, PPARgamma ligands have been reported to exert anti-inflammatory activity by inhibiting inflammatory gene expression while PPARgamma agonists bind to PPARs.[12] Many phytochemicals, including auraptene, resveratrol, 6-shogaol, and isoprenoid, are considered to function as PPARgamma agonists and demonstrate anti-inflammatory activity by interfering with nuclear factor-kappa B (NFκB) signaling.[13] Several flavonoids, such as rutin and quercetin, elevate PPARgamma mRNA expression, which attenuates inflammation and insulin resistance.[14,15] The transcriptional activity of PPARgamma is modulated through phosphorylation by kinases such as c-Jun N-terminal kinases (JNK). PPARgamma

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loses its transcriptional activity by JNK phosphorylation at serine 82, and is subsequently degraded by the ubiquitin pathway. Treating diabetes with PPARgamma ligands (agonists), such as pioglitazone, can prevent PPARgamma phosphorylation by altering its structure.[16]

PPARgamma is expressed in islet beta cells[17] and is important for a variety of pancreatic functions, including beta cell survival,[18] pancreatic and duodenal homeobox-1 (PDX-1) and glucokinase (GCK) regulation,[19] and glucose-stimulated insulin secretion.[20] In addition, PPARgamma is known to affect pancreatic beta cell function and insulin production.[21] Studies have reported that PPARgamma binds to the PDX-1 promoter to upregulate PDX-1 expression and insulin production.[19] A recent acute study suggested that AGE injection can initiate beta cell dysfunction and demonstrated that dietary restriction of AGEs significantly improves insulin sensitivity.[22] AGEs also decrease insulin synthesis in pancreatic beta cells by repressing PDX-1 protein expression and inhibiting glucose-stimulated insulin secretion.[23] PDX-1 plays a significant role in both pancreatic development and maintenance of beta cell function, but the inhibition of beta cell function caused by AGEs was improved by pioglitazone (PPARgamma agonist) activating PPARgamma.[24] Several lines of evidence indicate that PDX-1 binds to insulin and GCK and that GCK catalyzes the first step of glycolysis to regulate glucose

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104 responsiveness for insulin release.[25]

105 These findings indicated that PPARgamma plays an important role for diabetes
106 improvement. However, we had found that monascin is a PPARgamma agonist to
107 up-regulate insulin sensitivity and inhibited hyperglycemia in AGEs- or MG-treated
108 animals in our recent studies.

109

110 **2. Anti-inflammation and antioxidation of monascin**

111 High carbohydrate diets result in hyperglycemia and insulin resistance. In
112 diabetic patients, there is a positive correlation between high methylglyoxal (MG)
113 concentration in the blood and hyperglycemia. Recent studies have shown that MG
114 administration results in inflammation.[26]

115 Several literatures have reported the modulation of inflammatory cytokines
116 through oxidative stress.[27,28] Oxidative stress is increased during diabetes and
117 hyperinsulinemia; reactive oxygen species have been reported to be generated as a
118 result of hyperglycemia, which causes many of the secondary complications of
119 diabetes.[28]

120 We have indicated that monascin can suppress the production of inflammatory
121 factors (tumor necrosis factor-alpha and interleukin-6) from monocytes induced by
122 MG depending on PPARgamma regulation and these effects are abolished by

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PPARgamma inhibitor GW9662.[29] In addition, the anti-inflammatory capacity of monascin is mediated by the inhibition of JNK, extracellular signal-regulated kinase (ERK), and p38 kinases **(Fig. 2)**.[30]

Inflammation is an independent risk factor of cardiovascular diseases and is associated with endothelial dysfunction. *Monascus*-fermented metabolites, including monascin, ankaflavin, and monacolin K, have been found to reduce TNF-α-stimulated endothelial adhesiveness as well as downregulating intracellular ROS formation, NF-κB activation, and VCAM-1/E-selectin expression in human aortic endothelial cells, supporting the notion that the various metabolites from *Monascus*-fermented products might have potential implications in clinical atherosclerosis disease.[31]

Recently, our study also reports that monascin can extend the life span under high-glucose conditions and attenuate oxidative stress in *Caenorhabditis elegans*. Our results indicate that monascin enhanced expression of small heat shock protein (sHSP-16), superoxide dismutase (SOD), and glutathione S-transferase (GST). Monascin not only regulates stress response/antioxidant genes to improve oxidative stress resistance but also promotes antioxidation and avoid oxidative damage via regulation of the FOXO/DAF-16-dependent insulin signaling pathway.[32]

Moreover, Nrf2 has been found to attenuate oxidative damage by expressions of heme oxygenase-1 (HO-1), and glutathione-cysteine ligase (GCL).[33] Our study has

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carried out the Nrf2 regulation by monascin in vivo and in vitro. Results indicated that monascin inhibited inflammatory cytokine production in S100b (the receptor for AGEs activator)-treated THP-1 monocytes via up-regulation of Nrf2 and alleviated p47phox translocation to the membrane; and these effect were abolished by Nrf2 inhibitor treatment depending on retinoic acid receptor-alpha.[29] We also found that monascin markedly activated Nrf2 and attenuated insulin resistance in vitro and in vivo pointing out as **Fig. 3**.[26,29] These findings had pointed out that monascin suppressed oxidative stress and inflammation by showing antioxidation.

3. Anti-diabetic effect of monascin

Diabetes mellitus, which is characterised by hyperglycemia, is an endocrine disorder resulting from insulin deficiency that leads to high blood glucose concentration.[34] Type 2 diabetes and obesity are chronic diseases that promote the development of insulin resistance, inflammation, and atherosclerosis.[35] Type 2 diabetes is a chronic disease caused by deficient insulin secretion or ineffective insulin activity, thereby negatively affecting carbohydrate metabolism.[36] High triacylglycerol levels in the blood tend to coexist with low levels of high-density lipoprotein cholesterol (HDL-C), contributing to a condition called diabetic dyslipidemia or hypertriglyceridemia.[37] The total cholesterol (TC) and total

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161 triacylglycerol (TG) cause an increased risk of heart disease, which should be
162 controlled as tightly as possible in diabetes mellitus.[38] Insulin resistance in type 2
163 diabetic patients is thought to be associated with the induction of inflammatory
164 cytokines such as TNF-alpha and IL-6.[39] The TNF-alpha impairs insulin-dependent
165 signal transduction through a mechanism involving downregulation of the insulin
166 receptor (IR) and IR substrate-1 protein (IRS-1), inhibition of IR and IRS-1 tyrosine
167 phosphorylation, increased protein tyrosine phosphatase 1B (PTP1B) activity, and
168 inhibition of the insulin-stimulated glucose transporter (GLUT), thereby resulting in
169 hyperglycemia.[38] Results of our recent study have shown that monascin can attenuate
170 JNK phosphorylation and suppress PPARgamma phosphorylation in C2C12
171 myotubes treated with TNF-alpha and thereby improve insulin sensitivity.[40] In
172 addition, monascin also inhibits protein tyrosine (Tyr) phosphatase 1B (PTP1B)
173 expression to attenuate insulin resistance, resulting in GLUT translocation to plasma
174 membrane and subsequently promoting glucose uptake as shown in **Fig. 4**.[40]

175 In vitro studies suggest that MG impairs insulin mediated glucose uptake in
176 adipocytes[41] and reduces insulin sensitivity for 30 min in L6 muscle cells treated with
177 2.5 mM MG.[42] Moreover, 1 mM MG suppresses insulin secretion and production in
178 INS-1E pancreatic islet β-cells.[43] In vivo studies demonstrate that MG impairs insulin
179 transcription factor pancreatic and duodenal homeobox-1 (PDX-1) to result in

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diabetes.[44,45]

Recently, monascin has been reported to act as PPARgamma agonist,[8] and the in vitro (MG-treated RIN-m5F cells) and in vivo (MG-treated Balb/c mice) results indicated that MG leads to marked PPARgamma phosphorylation (serine 82); this effect led to reduction in PDX-1, GCK, and insulin expression. Monascin and rosiglitazone protected impairment of insulin expression in MG-treated animals confirmed by immunohistochemical stain for pancreatic insulin **(Fig. 5)**.[26] Moreover, monascin also prevented hyperglycemia and significantly downregulated blood glucose during oral glucose tolerance test (OGTT) in fructose-rich diet-induced C57BL/6 mice, and the potential mechanism was shown as **Fig. 6**.[46]

Hepatic stellate cells (HSCs) express the receptor for AGEs (RAGE)[47] and also express many components of the NADPH oxidase complex, such as p47phox. Importantly, one study has implicated p47phox-derived reactive oxygen species (ROS) in HSCs activation, suggesting that hepatic fibrosis is always involved in diabetes.[48] To gain better insights into the role of AGEs in HSCs, we investigated the effect of AGEs on ROS production by HSCs. Carboxymethyllysine (CML) is a key AGE with highly reactive dicarbonyl metabolites (e.g., methylglyoxal) and promotes lipid peroxidation to generate malondialdehyde (MDA).[49] We had investigated the inhibitory effect of *Monascus*-fermented metabolite monascin on CML-induced

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199 RAGE signaling in HSCs and its resulting antihepatic fibrosis activity. We found that
200 monascin upregulated PPARgamma to attenuate alpha-smooth muscle actin
201 (alpha-SMA) and ROS generation in CML-treated HSCs in a RAGE
202 activation-independent pathway. Therefore, monascin may regulate PPARgamma to
203 delay or inhibit the progression of liver fibrosis and may prove to be a major
204 antifibrotic mechanism to prevent liver disease **(Fig. 7)**.[50]

205

206 **4. Conclusions**

207 These health-promoting functions of monascin may be used to augment the
208 anti-metabolic syndrome, antihypertensive and anti-atherogenic effects of current
209 pharmacotherapeutics. The bioactivity of monascin is responsible for the previously
210 described health benefits and for the prevention of numerous inflammation-related
211 diseases. Together, these findings suggest that monascin can act as an antidiabetic and
212 antioxidative stress agent, and thus, monascin may have therapeutic potential in the
213 treatment or prevention of diabetes and diabetes-associated oxidative stress
214 complications.

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Conflict of interest

The authors declare that there are no conflicts of interest.

Food & Function Accepted Manuscript

References

1. M. van Tieghem, *Monascus* genre nouvear de l'ondre des Ascomycetes. *Bull de la Société Botanique de France,*1884, **31,** 226-231.
2. D. L. Hawksworth and J. I. Pit, A new taxonomy for *Monascus* species based on cultural and microscopical characters. *Aus. J. Botany,* 1983, **31,** 51-61.
3. T. Akihisa, H. Tokuda, M. Ukiya, A. Kiyota, K. Yasukawa, N. Sakamoto, Y. Kimura, T. Suzuki, J. Takayasu and H. Nishino, Anti-tumor initiating effects of monascin, an azaphilonoid pigment from the extract of *Monascus pilosus* fermented rice (red-mold rice). *Chem. Biodiv.,* 2005, **2,** 1305-1309.
4. T. Akihisa, H. Tokuda, K. Yasukawa, M. Ukiya, A. Kiyota, N. Sakamoto, T. Suzuki, N. Tanabe and H. Nishino, Azaphilones, furanoisophthalides, and amino acids from the extracts of *Monascus pilosus*-fermented rice (red-mold rice) and their chemopreventive effects. *J. Agric. Food Chem.,* 2005, **53,** 562-565.
5. Y. Kohama, S. Matsumoto, T. Mimura, N. Tanabe, A. Inada and T. Nakanishi, Isolation and identification of hypotensive principles in red mold rice. *Chem. Pharmaceut. Bull.,* 1987, **35,** 2484-2489.
6. L. Martinkova, P. Patakova-Juzlova, V. Kren, Z. Kucerova, V. Havlicek, P. Olsovsky, O. Hovorka, B. Rihova, D Vesely, D. Vesela, J. Ulrichova and V. Prikrylova, Biological activities of oligoketide pigments of *Monascus purpureus*.

Food & Function Accepted Manuscript

Food Addit. Contamin., 1999, **16,** 15-24.

7. B. H. Lee, W. H. Hsu, Y. W. Hsu and T. M. Pan, Dimerumic acid attenuates receptor for advanced glycation endproducts signal to inhibit inflammation and diabetes mediated by Nrf2 activation and promotes methylglyoxal metabolism into D-lactic acid. *Free Radic. Biol. Med.,* 2013, **60,** 7-16.

8. W. H. Hsu, B. H, Lee, Y. Y. Chang, Y. W. Hsu and T. M. Pan, A novel natural Nrf2 activator with PPAR-gamma agonist (monascin) attenuates the toxicity of methylglyoxal and hyperglycemia. *Toxicol. Appl. Pharmacol.,* 2013, **272,** 842-851.

9. J. F. Day, S. R. Thorpe and J. W. Baynes, Nonenzymatically glucosylated albumin: in vitro preparation and isolation from normal human serum. *J. Biol. Chem.,* 1979, **254,** 595-597.

10. C. H. Wu, S. M. Huang, J. A. Lin and G. C. Yen, Inhibition of advanced glycation endproduct formation by foodstuffs. *Food Funct.,* 2011, **2,** 224-234.

11. A. A. Bulhak, C. Jung, C. C. Ostenson, J. O. Lundberg, P. O. Sjoquist and J. Pernow, PPARγ activation protects the type 2 diabetic myocardium against ischemia-reperfusion injury: involvement of the PI3-kinas/Akt and NO pathway. *Am. J. Physiol. Heart. Circ. Physiol.,* 2009, **296,** H719-H727.

12. W. E. Ackerman, X. L. Zhang, B. H. Rovin and D. A. Kniss, Modulation of

Food & Function Accepted Manuscript

cytokine-induced cyclooxygenase 2 expression by PPARG ligands through NFkappaB signal disruption in human WISH and amnion cells. *Biol. Reprod.,* 2005, **73,** 527-535.

13. S. Hirai, N. Takahashi, T. Goto, S. Lin, T. Uemura, R. Yu and T. Kawada, Functional food targeting the regulation of obesity-induced inflammatory responses and pathologies. *Mediators Inflam.,* 2010, Doi:10.1155/2010/367838.
14. I. Choi, Y. Park, H. Choi and E. H. Lee, Anti-adipogenic activity of rutin in 3T3-L1 cells and mice fed with high-fat diet. *BioFactors,* 2006, **26,** 273-281.
15. C. C. Chuang, K. Martinez, G. Xie, A. Kennedy, A. Bumrungpert, A. Overman, W. Jia and M. K. Mclntosh, Quercetin is equally or more effective than resveratrol in attenuating tumor necrosis factor-α-mediated inflammation and insulin resistance in primary human adipocytes. *J. Am. Clin. Nutr.,* 2010, **92,** 1511-1521.
16. J. H. Choi, A. S. Banks, J. L. Estall, S. Kajimura, P. Bostrom, D. Laznik, J. L. Ruas, M. J. Chalmers, T. M. Kamenecka, M. Bluher, P. R. Griffin and B. M. Spiegelman, Anti-diabetic drugs inhibit obesity-linked phosphorylation of PPARγ by Cdk5. *Nature,* 2010, **466,** 451-456.
17. M. Dubois, F. Pattou, J. Kerr-Conte, V. Gmyr, B. Vandewalle, P. Desreumaux, J. Auwerx, K. Schoonjans and J. Lefebvre. Expression of peroxisome

Food & Function Accepted Manuscript

proliferator-activated receptor gamma (PPAR-gamma) in normal human pancreatic islet cells. *Diabetologia,* 2000, **43,** 1165-1169.

18. C. Y. Lin, T. Gurlo, L. O. Haataja, W. A. Hsueh and P. C. Butler, Activation of peroxisome proliferator-activated receptor-gamma by rosiglitazone protects human islet cells against human islet amyloid polypeptide toxicity by a phosphatidylinositol 3′-kinase-dependent pathway. *J. Clin. Endocrinol. Metab.,* **90,** 2005, 6678-6686.

19. J. A. Moibi, D. Gupta, T. L. Jetton, M. Peshavaria, R. Desai and J. L. Leahy, Peroxisome proliferator-activated receptor-γ regulates expression of PDX-1 and NKX6.1 in INS-1 cells. *Diabetes,* 2007, **56,** 88-95.

20. H. I. Kim, J. W. Kim, S. H. Kim, J. Y. Cha, K. S. Kim and Y. H. Ahn, Identification and functional characterization of the peroxisomal proliferator response element in rat GLUT2 promoter. *Diabetes,* 2000, **49,** 1517-1524.

21. N. Irwin, J. M. McKinney, C. J. Bailey, N. H. McClenaghan and P. R. Flatt, Acute and long-term effects of peroxisome proliferatoractivated receptor-γ activation on the function and insulin secretory responsiveness of clonal beta-cells. *Horm. Metab. Res.,* 2011, **43,** 244-249.

22. M. T. Coughlan, F. Y. T. Yap, D. C. K. Yong, S. Andrikopoulos, A. Gasser, V. Thallas-Bonke, D. E. Webster, J. I. Miyazaki, T. W. Kay, R. M. Slattery, D. M.

Food & Function Accepted Manuscript

297 Kaye, B. G. Drew, B. A. Kingwell, S. Fourlanos, P. H. Groop, L. C. Harrison, M.
298 Knip and J. M. Forbes, Advanced glycation end products are direct modulators of
299 beta-cell function. *Diabetes,* 2011, **60,** 2523-2532.
300 23. T. Shu, Y. Zhu, H. Wang, Y. Lin, Z. Ma and X. Han, AGEs decrease insulin
301 synthesis in pancreatic beta-cell by repressing PDX-1 protein expression at the
302 post-translational level. *PLoS One,* 2011, **6,** Article ID e18782.
303 24. A. Puddu, R. Sanguineti, A. Durante and G. L. Viviani, Pioglitazone attenuates
304 the detrimental effects of advanced glycation end-products in the pancreatic beta
305 cell line HIT-T15. *Regul. Pept.,* 2012, **177,** 79-84.
306 25. H. Kaneto, T. Miyatsuka and D. Kawamori, PDX-1 and MafA play a crucial role
307 in pancreatic beta-cell differentiation and maintenance of mature beta-cell
308 function. *Endocr. J.,* 2008, **55,** 235-252.
309 26. W. H. Hsu, B. H. Lee, C. H. Li, Y. W. Hsu and T. M. Pan, Monascin and AITC
310 attenuate methylglyoxal-induced PPARgamma phosphorylation and degradation
311 through inhibition of the oxidative stress/PKC pathway depending on Nrf2
312 activation. *J. Agric. Food Chem.,* 2013, **61,** 5996-6006.
313 27. S. M. Huang, C. H. Wu and G. C. Yen, Effects of flavonoids on the expression of
314 the pro-inflammatory response in human monocytes induced by ligation of the
315 receptor for AGEs. *Mol. Nutr. Food Res.,* 2006, **50,** 1129-1139.

Food & Function Accepted Manuscript

316 28. N. Shanmugam, M. A. Reddy, M. Guha and R. Natarajan, High glucose-induced
317 expression of proinflammatory cytokine and chemokinegenes in monocytic cells.
318 *Diabetes,* 2003, **52,** 1256-1264.
319 29. B. H. Lee, W. H. Hsu, T. Huang, Y. Y. Chang, Y. W. Hsu and T. M. Pan, Effects
320 of monascin on anti-inflammation mediated by Nrf2 activation in advanced
321 glycation end product-treated THP-1 monocytes and methylglyoxal-treated
322 Wistar rats. *J. Agric. Food Chem.,* 2013, **61,** 1288-1298.
323 30. W. H. Hsu, B. H. Lee, T. H. Liao, Y. W. Hsu and T. M. Pan, *Monascus*-fermented
324 metabolite monascin suppresses inflammation via PPARγ regulation and JNK
325 inactivation in THP-1 monocytes. *Food Chem. Toxicol.,* 2012, **50,** 1178-1186.
326 31. C. P. Lin, Y. L. Lin, P. H. Huang, H. S. Tsai and Y. H. Chen, Inhibition of
327 endothelial adhesion molecule expression by *Monascus purpureus*-fermented
328 rice metabolites, monacolin K, ankaflavin, and monascin. *J. Sci. Food Agric.,*
329 2011, **91**, 1751-1758.
330 32. Y. C. Shi, H. C. Liao and T. M. Pan, Monascin from red mold dioscorea as a
331 novel antidiabetic and antioxidative stress agent in rats and *Caenorhabditis*
332 *elegans*. *Free Radic. Biol. Med.,* 2012, **52,** 109-117.
333 33. D. V. Chartoumpekis, P. G. Ziros, A. I. Psyrogiannis, A. G. Papavassiliou, V. E.
334 Kyriazopoulou, G. P. Sykiotis and I. G. Habeos, Nrf2 represses FGF21 during

Food & Function Accepted Manuscript

long-term high-fat diet-induced obesity in mice. *Diabetes,* 2011, **60,** 2465-2473.

34. S. K. Singh, P. K. Rai, D. Jaiswal and G. Watal, Evidence-based critical evaluation of glycemic potential of *Cynodon dactylon*. *Evidence-Based Compl. Alter. Med.,* 2008, **5,** 415-420.

35. A. C. Thirone, C. Huang and A. Klip, Tissue-specific roles of IRS proteins in insulin signaling and glucose transport. *Trends Endocrinol. Metab.,* 2006, **17,** 72-78.

36. K. F. Petersen and G. L. Shulman, Etiology of insulin resistance. *Am. J. Med.,* 2006, **119,** S10-S16.

37. S. L. Abbate and J. D. Brunzell, Pathophysiology of hyperlipidemia in diabetes mellitus. *J. Cardiovasc. Pharmacol.,* 1990, **16,** S1-S7.

38. K. Ravi, B. Ramachandran and S. Subramanian, Protective effect of *Eugenia jambolana* seed kernel on tissue antioxidants in streptozotocin induced diabetic rats. *Biol. Pharmaceut. Bull.,* 2004, **27,** 1212-1217.

39. A. T. Cheung, J. Wang, D. Ree, J. K. Koll and M. Bryer-Ash, Tumor necrosis factor-α induces hepatic insulin resistance in obese zucker (fa/fa) rats via interaction of leukocyte antigen-related tyrosine phosphatase with focal adhesion kinase. *Diabetes,* 2000, **49,** 810-819.

40. B. H. Lee, W. H. Hsu, T. H. Liao and T. M. Pan, The *Monascus* metabolite

Food & Function Accepted Manuscript

monascin against TNF-α-induced insulin resistance via suppressing PPARγ phosphorylation in C2C12 myotubes. *Food Chem. Toxicol.,* 2012, **49,** 2609-2617.

41. X. Jia, D. J. Olson, A. R. Ross and L. Wu, Structural and functional changes in human insulin induced by methylglyoxal. *FASEB J.,* 2006, **20,** 1555-1557.

42. A. Riboulet-Chavey, A. Pierron, I. Durand, J. Murdaca, J. Giudicelli and E. Van Obberghen, Methylglyoxal impairs the insulin signaling pathways independently of the formation of intracellular reactive oxygen species. *Diabetes,* 2006, **55,** 1289-1299.

43. F. Fiory, A. Lombardi, C. Miele, J. Giudicelli, F. Beguinot and E. Van Obberghen, Methylglyoxal impairs insulin signalling and insulin action on glucose-induced insulin secretion in the pancreatic beta cell line INS-1E. *Diabetologia,* 2011, **54,** 2941-2952.

44. A. Dhar, K. M. Desai and L. Wu, Alagebrium attenuates acute methylglyoxal-induced glucose intolerance in Sprague-Dawley rats. *Bri. J. Pharmacol.,* 2010, **159,** 166-175.

45. A. Dhar, I. Dhar, B. Jiang, K. M. Desai and L. Wu, Chronic methylglyoxal infusion by minipump causes pancreatic β-cell dysfunction and induces type 2 diabetes in Sprague-Dawley rats. *Diabetes,* 2011, **60,** 899-908.

Food & Function Accepted Manuscript

46. B. H. Lee, W. H. Hsu, T. Huang, Y. Y. Chang, Y. W. Hsu and T. M. Pan, Monascin improves diabetes and dyslipidemia by regulating PPARgamma and inhibiting lipogenesis in fructose-rich diet-induced C57BL/6 mice. *Food Funct.,* 2013, **4**, 950-959.

47. H. Fehrenbach, R. Weiskirchen, M. Kasper and A. M. Gressner, Up-regulated expression of the receptor for advanced glycation end products in cultured rat hepatic stellate cells during transdifferentiation to myofibroblasts. *Hepatology,* 2001, **34,** 943-952.

48. E. L. M. Guimaraes, C. Empsen, A. Geerts and L. A. van Grunsven, Advanced glycation end products induce production of reactive oxygen species via the activation of NADPH oxidase in murine hepatic stellate cells. *J. Hepatol.,* 2010, **52,** 389-397.

49. T. M. Buetler, E. Leclerc, A. Baumeyer, H. Latado, J. Newell, O. Adolfsson, V. Parisod, J. Richoz, S. Maurer, F. Foata, D. Piguet, S. Junod, C. W. Heizmann and T. Delatour, N-Carboxymethyllysinemedified proteins are unable to bind to RAGE and activate an inflammatory response. *Mol. Nutr. Food Chem.,* 2008, **52,** 370-378.

50. W. H. Hsu, B. H. Lee, Y. W. Hsu and T. M. Pan, Peroxisome proliferator-activated receptor-gamma activators monascin and rosigliatazone

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392 attenuate carboxymethyllysine-induced fibrosis in hepatic stellate cells through
393 regulating the oxidative stress pathway but independent of the receptor for
394 advanced glycation endproducts signaling. *J. Agric. Food Chem.,* 2013, **61**,
395 6873-6879.

396

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397 **Figure legends**

398 **Figure 1.** (a) Chemical tructure of monascin. (b) Monascin is a PPARgamma
399 agonist.[8] The PPARgamma agonist activity of monascin was carried out by
400 LanthaScreen™ TR-FRET PPARγ coactivator assay kit (Invitrogen, Carlsbad, CA,
401 USA). PPARgamma: peroxisome proliferator-activated receptor-gamma.

402

403 **Figure 2.** The proposed mechanism of monascin on inflammation in THP-1 cell.
404 Ovalbumin-induced inflammation was alleviated by monascin via inhibition of JNK
405 phosphorylation and regulation of PPARgamma.[30] MS: monascin. JNK: c-Jun
406 N-terminal kinases. ERK: extracellular signal-regulated kinase.

407

408 **Figure 3.** The potential mechanism of monascin attenuated inflammation caused by
409 RAGE activation. Monascin promotes Nrf2 activation to elevate antioxidant status,
410 thereby attenuating oxidative stress and inflammation caused by RAGE signal.[29] MS:
411 monascin. AGEs: advanced glycation endproducts. RAGE: receptor for AGEs. TNF-α:
412 tumor necrosis factor-alpha. IL-1β: interleukin-1beta. PKC: protein kinase C. Nrf2:
413 nuclear factor-erythroid 2-related factor 2. HO-1: heme oxygenase-1. GCL:
414 glutathione-cysteine ligase.

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Figure 4. The inhibition of insulin resistance in C2C12 myotubes treated by monascin.[40] IR: insulin receptor. IRS: insulin receptor substrate. GLUT: glucose transporter. TNF-α: tumor necrosis factor-alpha. PPARgamma: peroxisome proliferator-activated receptor-gamma.

Figure 5. Effects of monascin, rosiglitazone, AITC, or NAC treatment on pancreatic insulin level of methylglyoxal-injected Balb/C mice stained by immunohistochemical stain.[26] Monascin promoted insulin expression and may protect impairment of pancreatic funtion in methylglyoxal-treated animals. MG: methylglyoxal. MS: monascin. Rosi: rosiglitazone. AITC: allyl isothiocyanate. NAC: N-acetylcysteine.

Figure 6. The potential anti-diabetic mechanism of monascin in mice fed high fructose diet.[46] Monascin improved fructose-rich diet-induced glucose intolerance, hyperlipidemia, hyperinsulinemia, and hepatic fatty acid accumulation, presumably by inhibiting lipogenesis and ameliorating insulin resistance and inflammation in the liver through PPARgamma activation. PPARgamma: peroxisome proliferator-activated receptor-gamma. ChREBP: carbohydrate responsive element binding protein. SREBP-1c: sterol regulatory element-binding protein-1c. ACC:

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435 acetyl-coA carboxylase. FAS: fatty acid synthase. PGC: peroxisome
436 proliferator-activated receptor-gamma coactivator.

437

438 **Figure 7.** Potential mechanism of monascin on antifibrosis in HSCs. Monascin and
439 rosiglitazone upregulated PPARgamma to attenuate fibrotic biomarker expression and
440 ROS generation in CML-treated HSCs.[50] CML: carboxymethyllysine. ROS: reactive
441 oxygen species. RAGE: receptor for advanced glycation endproducts. α-SMA:
442 α-smooth muscle actin. TIMP: tissue inhibitor of metalloproteinase. MMP-13: matrix
443 metalloproteinase-13.

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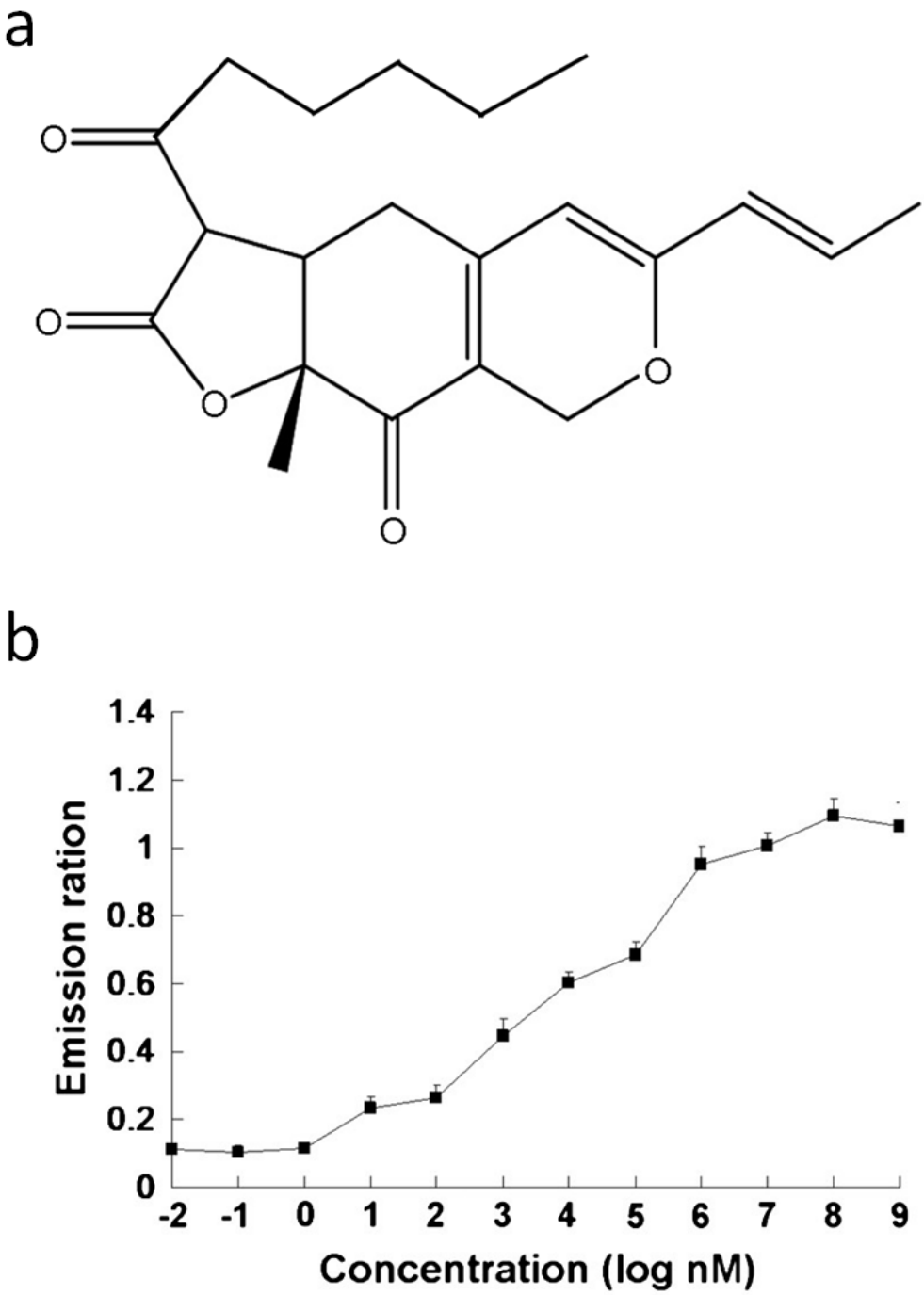

445

446 **Fig. 1**

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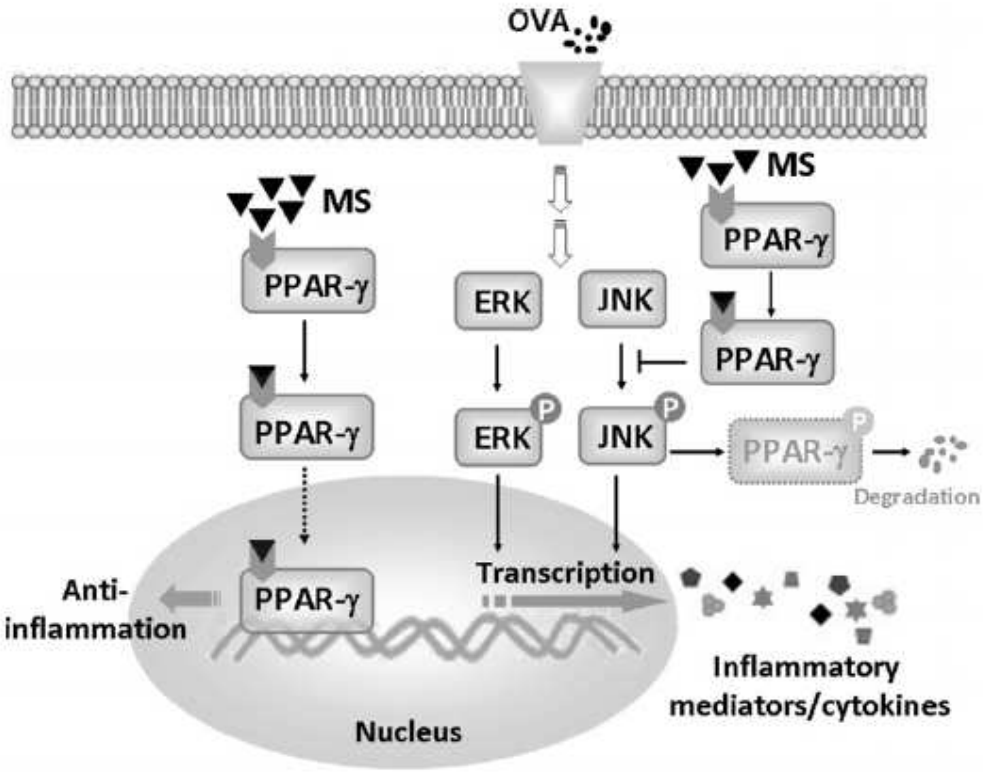

448

449 **Fig. 2**

450

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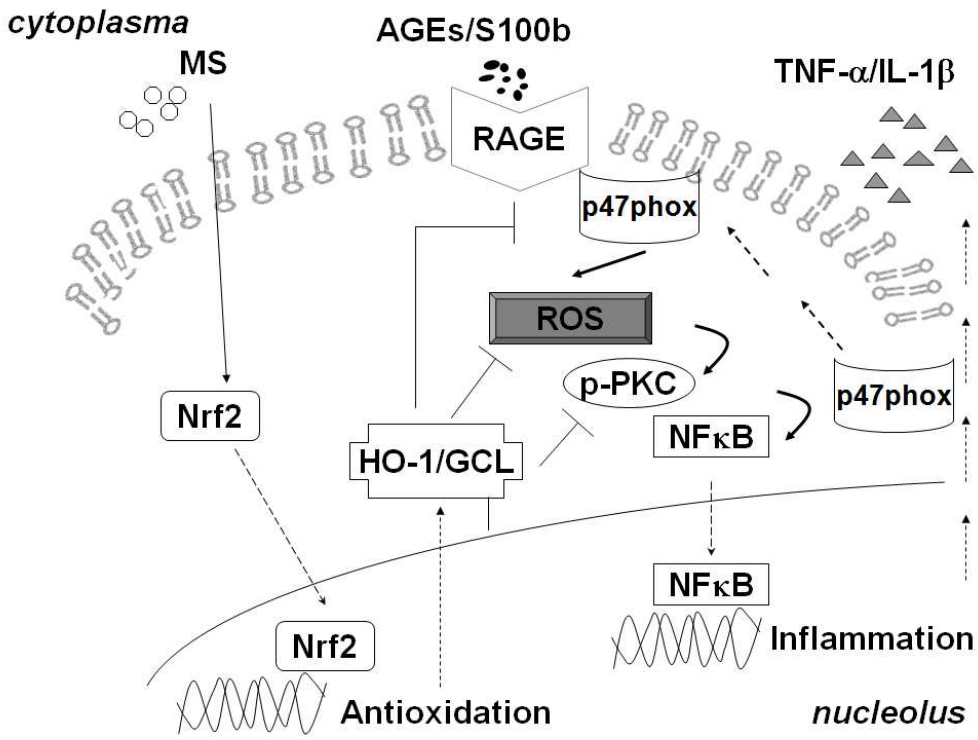

451

452 **Fig. 3**

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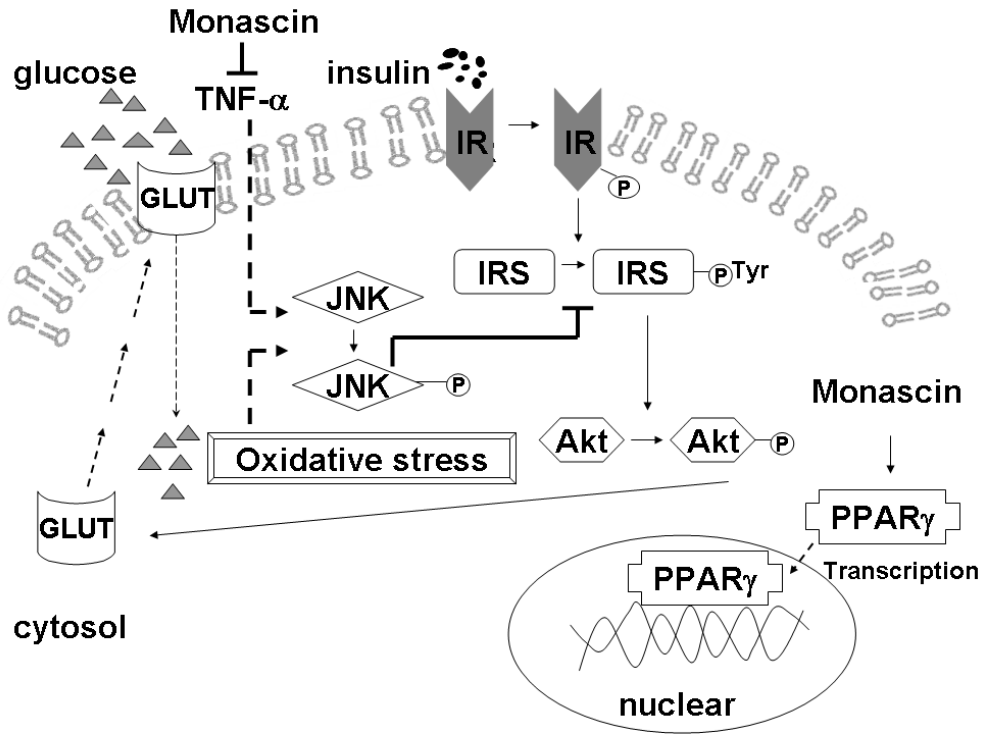

455

456 **Fig. 4**

457

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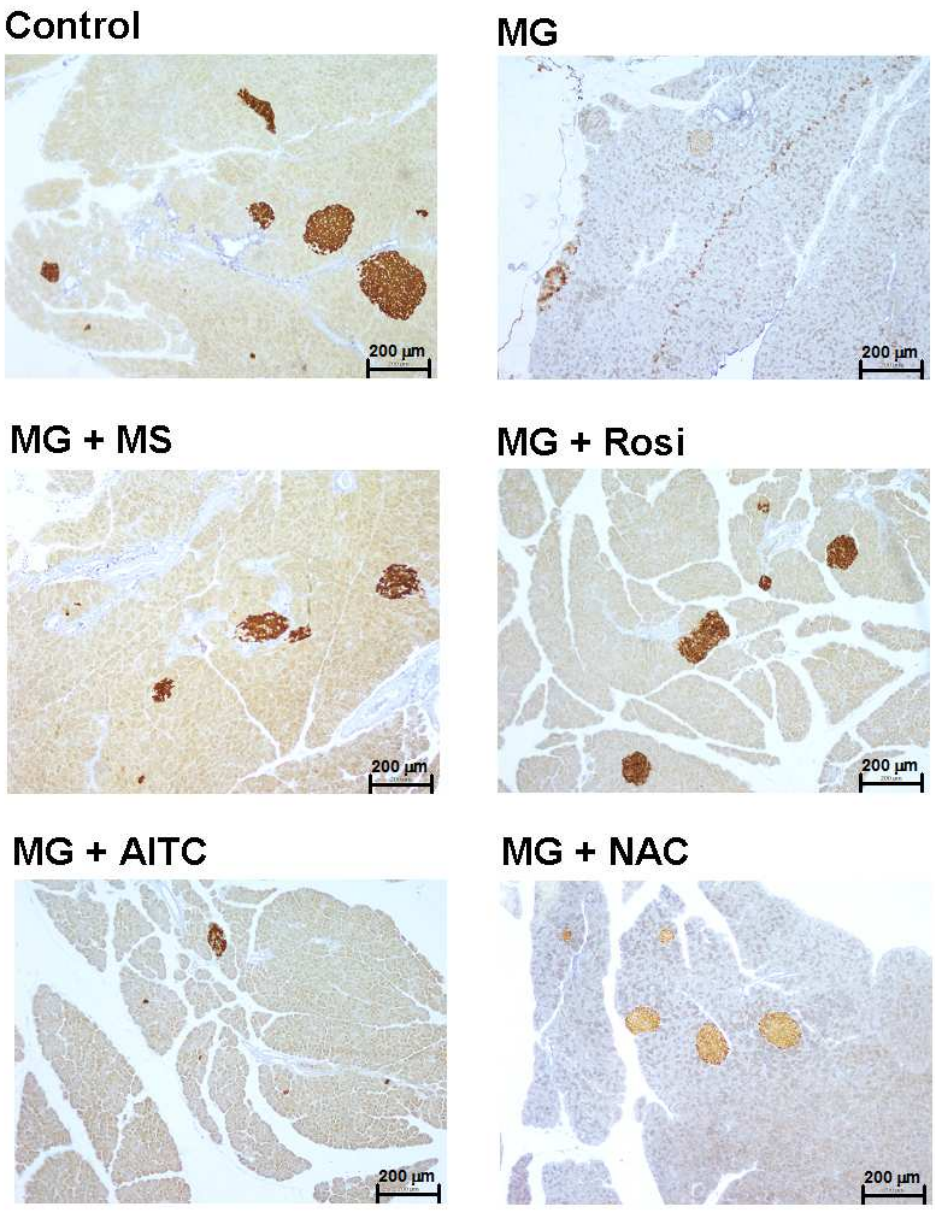

458
459 **Fig. 5**
460

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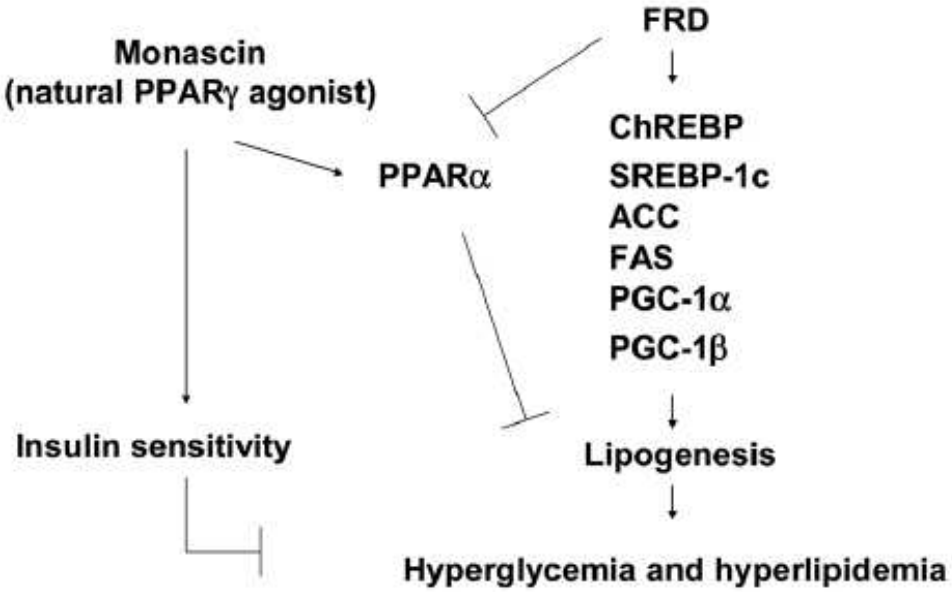

461

462 **Fig. 6**

463

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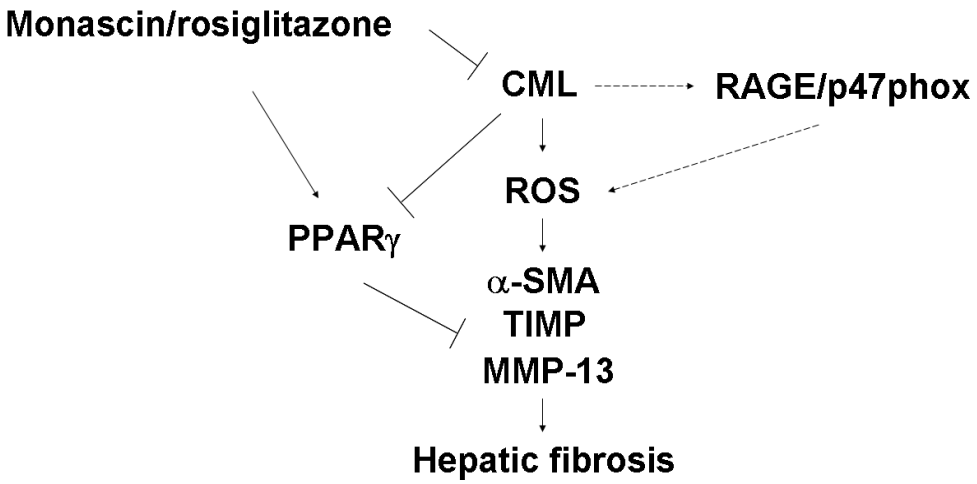

464

465 **Fig. 7**

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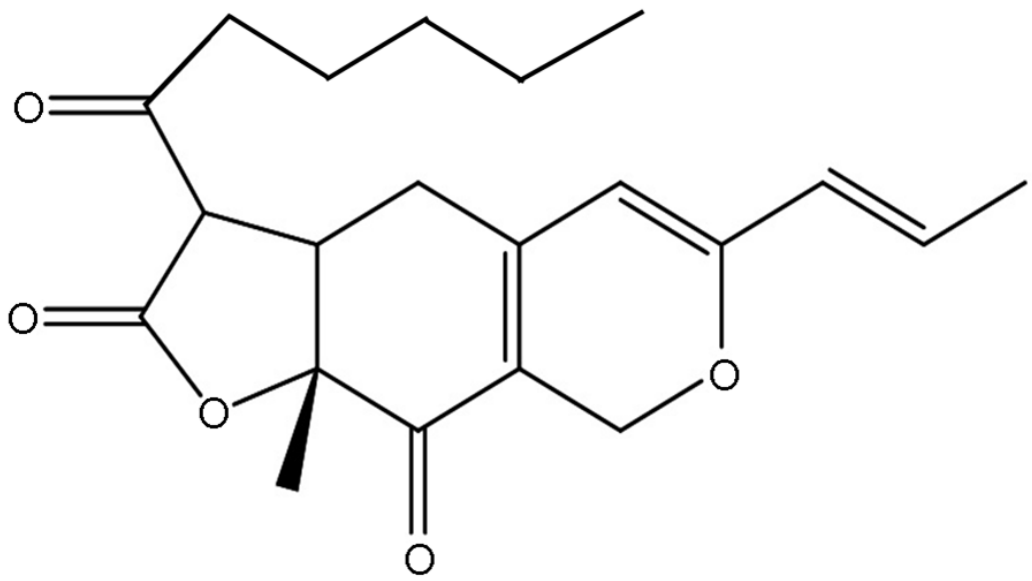

Graphical abstract

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