NPC Natural Product Communications

2014
Vol. 9
No. 9
1291 - 1294

Potent SIRT1 Enzyme-stimulating and Anti-glycation Activities of Polymethoxyflavonoids from *Kaempferia parviflora*

Asami Nakata[a], Yuka Koike[b], Hirofumi Matsui[c], Tsutomu Shimada[d], Masaki Aburada[d] and Jinwei Yang[a,*]

[a]*Tokiwa Phytochemical Co. Ltd.*, *158 Kinoko*, *Sakura*, *Chiba 285-0801*, *Japan*

[b]*Division of Pharmacognosy & Phytochemistry Department of Medicinal Chemistry*, *Showa University*, *1-5-8 Hatanodai*, *Shinagawa-ku*, *Tokyo 142-8555*, *Japan*

[c]*Division of Clinical Medicine*, *Faculty of Medicine*, *University of Tsukuba*, *1-1-1 Tennnodai*, *Tsukuba-shi*, *Ibaraki 305-8575*, *Japan*

[d]*Research Institute of Pharmaceutical Science*, *Musashino University*, *1-1-20 Shimachi*, *Nishitokyo-shi*, *Tokyo 202-8585*, *Japan*

k-you@tokiwaph.co.jp

Received: April 2nd, 2014; Accepted: June 1st, 2014

The SIRT1 enzyme-stimulating and anti-glycation activities of *Kaempferia parviflora* extract and its main polymethoxyflavonoids were evaluated *in vitro*. *K. parviflora* extract elevated SIRT1 catalytic activity by eight- and 17-fold at 20 μg/mL and 100 μg/mL, respectively, compared with vehicle only. Two major polymethoxyflavonoids, 3,5,7,3',4'-pentamethoxyflavone (**4**) and 5,7,4'-trimethoxyflavone (**5**), were isolated from this extract and are four- and fivefold more potent than resveratrol, hitherto the strongest known natural SIRT1 activator. In addition, the anti-glycation activity of *K. parviflora* extract was observed to be seven times more effective than aminoguanidine, a clinical anti-diabetes drug. 3,5,7,3',4'-Pentamethoxyflavone (**4**) and 5,7,4'-trimethoxyflavone (**5**) showed the strongest anti-glycation activity among the tested polymethoxyflavonoids. Further comparison of the activity of these structurally related polymethoxyflavonoids revealed a possible structure-activity relationship, in particular, for the contribution of methoxy moieties.

Keywords: *Kaempferia parviflora*, SIRT1, Advanced glycation end products, Polymethoxyflavonoids, Black turmeric.

Kaempferia parviflora Wall. ex. Baker, also called black turmeric or black ginger, is a plant belonging to the family *Zingiberaceae*. It is indigenous to southeast Asia, including Laos and Thailand, and has been used as a folk medicine to increase vitality, lower blood glucose levels, and provide nutrition. *K. parviflora* has been reported to have various biological properties, such as cholinesterase inhibitory [1], NO production-promoting [2], anti-inflammatory [3], anti-convulsion [4], anti-allergic [5], obesity prevention [6, 7], and gastric ulcer inhibition activities [8]. Polymethoxyflavonoids are typical components of *K. parviflora* [9], but they are not found in other *Zingiberaceae* species, such as *Curcuma longa* and *Zingiber officinale*.

Recently, Sirtuin 1 (SIRT1) has attracted much attention as a new longevity regulator. SIRT1, known as an NAD^+-dependent deacetylase, plays many important roles not only by deacetylating histones but also modulating activities of various transcription factors/coactivators, such as p53, NF-κB, and PGC-1α. It is expected that the activation of SIRT1 in the brain, liver, pancreas, muscle, and fat cells delays the onset of aging-related diseases and extends healthy life expectancy [10]. Resveratrol, a natural polyphenol in red wine, was reported to be the most potent natural stimulator of SIRT1 enzymatic action [11].

Glycation is a non-enzymatic and irreversible reaction between proteins and sugar to produce advanced glycation end products (AGEs). The accumulation of AGEs in skin leads to the browning of proteins and dulling of skin [12] and accumulation in blood vessels causes arteriosclerosis due to cross-bridge collagen [13]. Recently, it was also reported that AGEs are involved in the development of diabetic cardiomyopathy by decreasing the expression of SIRT1 [14].

In this paper, we reported the SIRT1 enzyme-stimulating and anti-glycation activities of a *K. parviflora* extract and the major polymethoxyflavonoids of this extract. The structure-activity relationship is also discussed.

We evaluated the stimulating activities of samples on the SIRT1 enzyme using human SIRT1 enzyme (SE-239) and a synthetic peptide substrate that contained lysine 382 of p53 *in vitro*. The *K. parviflora* extract concentration dependently elevated the SIRT1 catalytic activity eight- and 17-fold at the concentrations of 20 μg/mL and 100 μg/mL, respectively, compared with vehicle only. At the above concentrations, the potencies of *K. parviflora* extract are four- and five-fold those of resveratrol, the most potent natural SIRT1 activator [11] (Figure 1).

On the basis of the above results, the *K. parviflora* extract was further partitioned using water and EtOAc. The EtOAc fraction showed more potent SIRT1-stimulating activity, 16- and 23-fold that of vehicle only, at the concentrations of 20 μg/mL and 100 μg/mL. Further separation of this bioactive fraction by repeated column chromatography resulted in the isolation of five polymethoxyflavonoids: 3,5,7-trimethoxyflavone (**1**), 3,5,7,4'-tetramethoxyflavone (**2**), 5,7-dimethoxyflavone (**3**), 3,5,7,3',4'-pentamethoxyflavone (**4**), and 5,7,4'-trimethoxyflavone (**5**) (Figure 2). The structures were identified by detailed spectroscopic analysis and comparison with literature data [15]. These compounds are major components of *K. parviflora* extract, which was demonstrated by HPLC analysis and quantity determination of polymethoxyflavonoids in the *K. parviflora* extract. The contents of polymethoxyflavonoids **1**, **2**, **3**, **4**, and **5** in the *K. parviflora* extract were 0.9%, 2.2%, 4.2%, 4.5%, and 4.6%, respectively.

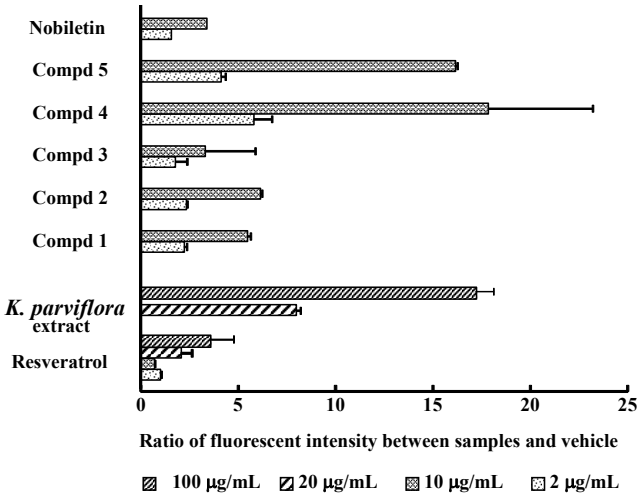
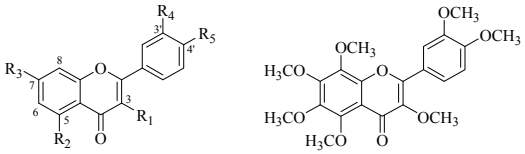


Figure 1: SIRT1 enzyme-stimulating activity of compounds **1-5**, *K. parviflora* extract, nobiletin and resveratrol (Mean ± SD, n=3).



No.	Compound	R_1	R_2	R_3	R_4	R_5
1	3,5,7-trimethoxyflavone	OCH_3	OCH_3	OCH_3	H	H
2	3,5,7,4'-tetramethoxyflavone	OCH_3	OCH_3	OCH_3	H	OCH_3
3	5,7-dimethoxyflavone	H	OCH_3	OCH_3	H	H
4	3,5,7,3',4'-pentamethoxyflavone	OCH_3	OCH_3	OCH_3	OCH_3	OCH_3
5	5,7,4'-trimethoxyflavone	H	OCH_3	OCH_3	H	OCH_3

Figure 2: Chemical structures of polymethoxyflavonoids **1-5** isolated from *K. parviflora* extract and nobiletin.

Polymethoxyflavonoids **1-5** were evaluated for their SIRT1-stimulating activities. The stimulating activity of the samples on the SIRT1 enzyme was calculated as the ratio of fluorescent intensity between samples and vehicle (Figure 1). At the concentrations of 2 μg/mL and 10 μg/mL, all compounds showed stronger stimulating activity than the positive control, resveratrol; compounds **4** and **5** were found to be the most active compounds and were four- and fivefold more potent than resveratrol at the concentration of 2 μg/mL. This is the first report on the SIRT1 stimulation activities of polymethoxyflavonoids. Some flavonoids, such as quercetin, have been reported to have SIRT1-stimulating activities, but are less effective than resveratrol [11]. This suggests that the presence of polymethoxy moieties is an important factor for SIRT1 stimulation.

Table 1: Anti-glycation activity of compounds **1-5** and nobiletin (Mean ± SD, n=3).

Compound	Anti-glycation activity (IC_{50}, μg/mL)
1	> 50
2	9.57±0.92
3	> 50
4	5.87±1.04
5	8.69±0.66
Nobiletin	> 50
Aminoguanidine	165.5±22.8

In addition, we also evaluated the anti-glycation activities of the *K. parviflora* extract and the aforementioned five polymethoxyflavonoids **1-5** *in vitro* (Table 1). The inhibition rate for the glycation reaction was determined by adding the samples to the reaction solution of human serum albumin and glucose and then comparing the AGE fluorescent intensity with the control. The IC_{50} value of *K. parviflora* extract was 25.1 μg/mL, which was seven times more effective than aminoguanidine (IC_{50} = 165.5 μg/mL), an anti-diabetes drug commonly used in the USA. Among the tested polymethoxyflavonoids, compounds **2**, **4**, and **5** showed the highest anti-glycation activities with IC_{50} values of 9.6, 5.9, and 8.7 μg/mL, respectively.

As shown in Table 1, compounds **1-5** showed different levels of activity as SIRT1 activators and glycation inhibitors, though they are structurally similar compounds. Compounds **4** and **5** were more potent SIRT1 stimulators and glycation inhibitors than the other polymethoxyflavonoids used in this study. To discuss the structure-activity relationship, we also evaluated the activity of a common polymethoxyflavonoid from citrus, nobiletin (5,6,7,8,3',4'-hexamethoxyflavone, Figure 2).

A comparison of the anti-glycation activity of compounds **5** and **3** suggested that the methoxy moiety at C-4' of ring B was essential for the observed activity. The methoxy moiety at C-3' of ring B only contributed to the activities to a small extent, as revealed by comparing the activities between compounds **4** and **2**. Furthermore, the polymethoxyflavonoid nobiletin, which has two additional methoxy moieties at C-6 and 8 of ring B compared with compound **4**, showed weak SIRT1-stimulating activity and no anti-glycation activity, suggesting that these two methoxy functions may play an important role in the down-regulation of activity. It has been reported that some antioxidants can scavenge the active oxygen radicals produced in the glycation process, and reduce the formation of reactive carbonyl compounds, leading to anti-glycation activity [17]. Polymethoxyflavonoids, which have been reported to have anti-oxidative activities [18], were suggested to interfere in the glycation reaction through a similar mechanism.

As a natural SIRT1 activator, resveratrol has attracted much attention, and many dietary supplements containing resveratrol have been developed due to its notable SIRT1-stimulating activity. Borra *et al*. previously reported that resveratrol with a coumarin-labelled peptide affected a conformational change near the coumarin-binding site in SIRT1, which led to an experimental artifact [19]. In addition, some compounds, such as SRT1460, SRT1720, and SRT2183, have also been described as SIRT1 enzyme-stimulating compounds [20]. However, these compounds were also reported not to be direct stimulators of SIRT1, because SIRT1 activation by these compounds was not observed in native peptides [21]. Therefore, it will be necessary to argue the *in vivo* potency of the isolated polymethoxyflavonoids to elicit SIRT1-dependent biological activities.

SIRT1 has been proven to regulate a number of targets involved in insulin resistance [22], vascular senescence [23], lipid metabolism [24], and inflammation [25]. Conversely, *K. parviflora* has been reported to and was found to improve metabolic syndrome in TSOD mice, a spontaneously obese Type II diabetes model [6, 7]. Anti-inflammatory activity was also observed for the *K. parviflora* extract [3]. It is reasonable to believe that the effects of *K. parviflora* include all of the above due to the SIRT1-stimulating activity of the isolated polymethoxyflavonoids.

In conclusion, we found that the *K. parviflora* extract showed potent SIRT1 enzyme-stimulating and anti-glycation activities, and polymethoxyflavonoids were identified as being responsible for these. To our knowledge, this is the first report of an SIRT1 enzyme-stimulating activity of polymethoxyflavonoids. Two polymethoxyflavonoids, 3,5,7,3',4'-pentamethoxyflavone (**4**) and 5,7,4'-trimethoxyflavone (**5**), showed the most potent activity,

suggesting their potential as lead compounds for new anti-aging drug development.

Experimental

General: The ^{1}H- and ^{13}C-NMR spectra were measured on a JEOL ECP-600 spectrometer with TMS as the internal reference. For isolation, a SHIMADZU LC-10A HPLC system equipped with a Shimadzu RID-10A refractive index detector and SPD-10Avp UV detector was used. Silica gel CC was carried out using Silica gel F (Shin-Etsu Kasei Kogyo Co., Ltd, Japan) and TLC used Silica gel 60 F254 plates (Merck). For analysis, a HITACHI ELITE LaChrom system equipped with diode array detector L-2450, pump L-2130, column oven L-2300, and auto-sampler L-2200 was used. Absorbance for bioactive assay was measured with a microplate reader, Tecan Infinite 200 (Tecan Japan Co., Ltd., Kanagawa, Japan). Nobiletin was purchased from Tokyo Kasei Kogyo Co., Ltd (Tokyo, Japan), and aminoguanidine hydrochloride from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

Plant material: *K. parviflora* (rhizome) was collected in 2009 in Thailand, and a voucher specimen was deposited at the herbal museum in Tokiwa Phytochemical Co., Ltd.

Preparation of K. parviflora extract: The dried rhizomes of *K. parviflora* (1 kg) were extracted with 80%, v/v, EtOH (10 L) by reflux, 2 times. The extraction solution was filtered, evaporated under vacuum, and mixed with dextrin to obtain the final dry extract powder (207.4 g).

Isolation of polymethoxyflavonoids: The above-obtained *K. parviflora* extract (10 g) was suspended in water (100 mL) and partitioned with EtOAc (100 mL) 3 times. The EtOAc fraction, which exhibited the most potent SIRT1-stimulating activity, was subjected to a silica gel column and eluted with a gradient of *n*-hexane-EtOAc in ratios from 90: 10 to 10: 90, to give 31 fractions. Further isolation of these fractions by preparative HPLC (Waters BONDASPHERE 5-μ C18-100A column, 150 × 19 mm i.d.) afforded **1** (14 mg), **2** (31 mg), **3** (253 mg), **4** (368 mg), and **5** (142 mg).

The ^{1}H and ^{13}C NMR spectroscopic data of compounds **1-5** were in good agreement with those of previously reported polymethoxyflavonoids: 3,5,7-trimethoxyflavone (**1**), 3,5,7,4'-tetramethoxyflavone (**2**), 5,7-dimethoxyflavone (**3**), 3,5,7,3',4'-pentamethoxyflavone (**4**), and 5,7,4'-trimethoxyflavone (**5**) (Figure 2) [15].

HPLC analysis and quantity determination of polymethoxyflavonoids in K. parviflora extract: HPLC analysis of the obtained *K. parviflora* extract was conducted using a Shiseido Capcell PAK C_{18} 250×4.6-mm i.d. column, eluted with 0.1% HCOOH-CH_3CN (70: 30) at a flow rate of 1.0 mL/min. The *K. parviflora* extract and each polymethoxyflavonoid standard compound were dissolved in 50%, v/v, EtOH and filtered through a 0.45-μm membrane filter. The detection wavelength was 260 nm. Peaks were assigned by comparing the retention times between the *K. parviflora* extract and standard compounds.

Assay of SIRT1 enzyme-stimulating activity: SIRT1 enzyme activity was measured using an SIRT1 Fluorimetric Drug Discovery kit (AK-555, BIOMOL International, Plymouth Meeting, PA, USA [COSMO BIO Co., Ltd., Tokyo]) according to the manufacturer's protocol. All reagents (storage at -80°C) were placed on ice until use. The 5 × Developer II (KI-176) and SIRT1 enzyme (SE-239) were slowly dissolved and the SIRT1 enzyme was diluted to 0.2 U/μL with assay buffer (KI-286). Sample solutions, at a concentration of 500 μg/mL in DMSO, were added at 10 μL/well, and the control was created by adding the vehicle (purified water or DMSO) instead of the test sample. Fluor de Lys-SIRT1, deacetylase substrate, (KI-177; 5 mM) and NAD+ (KI-282; 50 mM) were added at 15 μL/well, after being diluted to 3.33 times the final concentration. Each test sample solution (10 μL/well), SIRT1 enzyme solution (0.2 U/μL, 5 μL/well), and assay buffer (10 μL/well) were combined in a 96-well microplate, and the mixture was pre-incubated at 37°C for 5 min. The combined 25 μL/well substrate solution was kept at 37°C and further incubated at 37°C for 10 min. Finally, Developer II/2 mM nicotinamide (reaction stop solution) was added at 50 μL/well, and the fluorescence was measured using a fluorescence microplate reader within 60 min after stopping the reaction (excitation wavelength 360 nm; emission wavelength 460 nm). The stimulating activity of the samples on the SIRT1 enzyme was calculated as the ratio of fluorescent intensity between samples and control (vehicle). Resveratrol contained in the kit was used as SIRT1 activator.

Determination of anti-glycation activity: An *in vitro* glycation model was generated under the following conditions [16]. Briefly, the reaction solution containing 40 μL of 8 mg/mL human serum albumin (HSA; Sigma Chemical, MO, USA), 20 μL of 0.2 mo1/L glucose, 20 μL of each sample, and 100 μL of 0.05 mol/L phosphate buffer (PBS) (pH 7.4) was incubated at 60°C for 40 h. The samples were dissolved in DMSO and then diluted with water to the assigned concentrations. The fluorescence of AGEs was determined using the Infinite 200 microplate reader at an excitation wavelength of 370 nm and fluorescence wavelength of 440 nm.

The AGE production inhibition rate was calculated as below.

AGE production inhibition rate (%) = {1-(A-B)/(C-D)} ×100

A: Fluorescence intensity of reaction solution containing glucose and the sample.

B: Fluorescence intensity of reaction solution containing water instead of glucose and the sample.

C: Fluorescence intensity of reaction solution containing glucose and water instead of the sample.

D: Fluorescence intensity of reaction solution containing water instead of the sample and water instead of glucose.

IC_{50} (μg/mL) was calculated from the AGE production inhibition rate of each sample.

References

[1] Sawasdee P, Sabphon C, Sitthiwongwanit D, Kokpol U. (**2009**) Anticholinesterase activity of 7-methoxyflavones isolated from *Kaempferia parviflora*. *Phytotherapy Research*, ***23***, 1792–1794.

[2] Wattanapitayakul SK, Suwatronnakorn M, Chularojmontri L, Herunsalee A, Niumsakul S, Charuchongkolwongse S, Chansuvanich N. (**2007**) *Kaempferia parviflora* ethanolic extract promoted nitric oxide production in human umbilical vein endothelial cells. *Journal of Ethnopharmacology*, ***110***, 559–562.

[3] Sae-wong C, Tansakul P, Tewtrakul S. (**2009**) Anti-inflammatory mechanism of *Kaempferia parviflora* in murine macrophage cells (RAW 264.7) and in experimental animals. *Journal of Ethnopharmacology*, ***124***, 576–580.

[4] Wattanapitayakul SK, Chularojmontri L, Herunsalee A, Charuchongkolwongse S, Chansuvanich N. (**2008**) Vasorelaxation and antispasmodic effects of *Kaempferia parviflora* ethanolic extract in isolated rat organ studies. *Fitoterap*ia, ***79***, 214–216.
[5] Tewtrakul S, Subhadhirasakul S. (**2007**) Anti-allergic activity of some selected plants in the *Zingiberaceae* family. *Journal of Ethnopharmacology*, ***109***, 535–538.
[6] Akase T, Shimada T, Terabayashi S, Ikeya Y, Sanada H, Aburada M. (**2011**) Antiobesity effects of *Kaempferia parviflora* in spontaneously obese type II diabetic mice. *Journal of Natural Medicines*, ***65***, 73-80.
[7] Shimada T, Horikawa T, Ikeya Y, Matsuo H, Kinoshita K, Taguchi T, Ichinose K, Takahashi K, Aburada M. (**2011**) Preventive effect of *Kaempferia parviflora* ethyl acetate extract and its major components polymethoxyflavonoid on metabolic diseases. *Fitoterapia*, ***82***, 1272-1278.
[8] Rujjanawate C, Kanjanapothi D, Amornlerdpison D, Pojanagaroon S. (**2005**) Anti-gastric ulcer effect of *Kaempferia parviflora*. *Journal of Ethnopharmacology*, ***102***, 120–122.
[9] Horikawa T, Shimada T, Okabe Y, Kinoshita K, Koyama K, Miyamoto K, Ichinose K, Takahashi K, Aburada M. (**2012**) Polymethoxyflavonoids from *Kaempferia parviflora* induce adipogenesis on 3T3-L1 preadipocytes by regulating transcription factors at an early stage of differentiation. *Biological & Pharmaceutical Bulletin*, ***35***, 686-692.
[10] Feige JN, Auwerx J. (**2008**) Transcriptional targets of sirtuins in the coordination of mammalian physiology. *Current Opinion in Cell Biology*, ***20***, 303-309.
[11] Howitz KT, Bitterman KJ, Cohen HY, Lamming DW, Lavu S, Wood JG, Zipkin RE, Chung P, Kisielewski A, Zhang L, Scherer B, Sinclair DA. (**2003**) Small molecule activators of sirtuins extend *Saccharomyces cerevisiae* lifespan. *Nature*, ***425***, 191-196.
[12] Cerami A, Vlassara H, Brownlee M. (**1987**) Glucose and aging. *Scientific American*, ***256***, 90-96.
[13] Bastaa G, Schmidtb AM, Caterinaa RD. (**2004**) Advanced glycation end products and vascular inflammation: implications for accelerated atherosclerosis in diabetes. *Cardiovascular Research*, ***63***, 582-592.
[14] Yuan Q, Zhou QY, Liu D, Yu L, Zhan L, Li XJ, Peng HY, Zhang XL, Yuan XC. (**2014**) Advanced glycation end-products impair Na+/K+-ATPase activity in diabetic cardiomyopathy: role of the AMPK/SIRT1 pathway. *Clinical Experimental Pharmacology and Physiology*, ***41***, 127-133.
[15] Sutthanut K, Sripanidkulchai B, Yenjai C, Jay M. (**2007**) Simultaneous identification and quantitation of 11 flavonoid constituents in *Kaempferia parviflora* by gas chromatography. *Journal of Chromatography A*, ***1143***, 227–233.
[16] Hori M, Yagi M, Nomoto K, Ichijo R, Shimoda A, Kitano T, Yonei Y. (**2012**) Experimental models for advanced glycation end product formation using albumin, collagen, elastin, keratin and proteoglycan. *Anti-Aging Medicine*, ***9***, 125-134.
[17] Jariyapamornkoon N, Yibchok-anun S, Adisakwattana S. (**2013**) Inhibition of advanced glycation end products by red grape skin extract and its antioxidant activity. *BMC Complementary and Alternative Medicine*, ***13***, 171-179.
[18] Murakami A, Nakamura Y, Ohto Y, Yano M, Koshiba T, Koshimizu K, Tokuda H, Nishino H, Ohigashi H. (**2000**) Suppressive effects of citrus fruits on free radical generation and nobiletin, an anti-inflammatory polymethoxyflavonoid. *Biofactors*, ***12***, 187–192.
[19] Borra MT, Smith BC, Denu JM. (**2005**) Mechanism of human SIRT1 activation by resveratrol. *Journal of Biological Chemistry*, ***280***, 17187-17195.
[20] Milne JC, Lambert PD, Schenk S, Carney DP, Smith JJ, Gagne DJ, Jin L, Boss O, Perni RB, Vu CB, Bemis JE, Xie R, Disch JS, Ng PY, Nunes JJ, Lynch AV, Yang H, Galonek H, Israelian K, Choy W, Iffland A, Lavu S, Medvedik O, Sinclair DA, Olefsky JM, Jirousek MR, Elliott PJ, Westphal CH. (**2007**) Small molecule activators of SIRT1 as therapeutics for the treatment of type 2 diabetes. *Nature*, ***450***, 712-716.
[21] Pacholec M, Bleasdale JE, Chrunyk B, Cunningham D, Flynn D, Garofalo RS, Griffith D, Loulakis P, Pabst B, Qiu X, Stockman B, Thanabal V, Varghese A, Ward J, Withka J, Ahn K. (**2010**). SRT1720, SRT2183, SRT1460, and resveratrol are not direct activators of SIRT1. *Journal of Biological Chemistry*, ***285***, 8340-8351.
[22] Liang F, Kume S, Koya D. (**2009**) SIRT1 and insulin resistance. *Nature Reviews Endocrinology*, ***5***, 367-373.
[23] Ohta H. (**2007**) The mechanism of vascular senescence regulated by longevity gene, Sirt1. *Nippon Ronen Igakkai Zasshi*, ***44***, 194-197.
[24] Rodgers JT, Puigserver P. (**2007**) Fasting-dependent glucose and lipid metabolic response through hepatic sirtuin. *Proceedings of National Academy of Sciences USA*, ***104***, 12861–12866.
[25] Yeung F, Hoberg JE, Ramsey CS, Keller MD, Jones DR, Frye RA, Mayo MW. (**2004**) Modulation of NFκB-dependent transcription and cell survival by the SIRT1 deacetylase. *EMBO Journal*, ***23***, 2369–2380.