Review Article

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Piceatannol: A Potential Futuristic Natural Stilbene as Fetal Haemoglobin Inducer

Haematology Section

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ABSTRACT

Beta thalassaemia is an autosomal recessive inherited blood disorder which results in abnormal formation of Haemoglobin molecule and ineffective erythropoiesis. Patients need to be dependent on habitual blood transfusion and on unaffordable exorbitant therapies for continued existence. It has been hypothesized that if the level of foetal Haemoglobin increases, it compensates the need of adult Haemoglobin and hence, ameliorates clinical symptoms associated with beta thalassaemia major. Illation from previous studies has proved that reactivation of foetal Haemoglobin with the aid of natural compounds is a better alternative therapy for patients of beta thalassaemia because of its cost effectiveness and occurrence in natural eatables. Piceatannol, a naturally occurring stilbene, is less studied compound in comparison to resveratrol, but it shows a wide range of biological activities. This article has mainly focused on piceatannol and its application as a foetal Haemoglobin inducer in future.

Keywords: Beta thalassaemia, Foetal Haemoglobin inducer, Piceatannol, Stilbene

INTRODUCTION

Haemoglobinopathies, beta thalassaemia and sickle cell anaemia are genetic disorders that arise due to absence of globin gene or improper synthesis of Haemoglobin molecules in humans. These genetic diseases are a considerable cause of mortality around the world. Advancement in research methodologies is providing a novel approach for treatment of these disorders. Medical heterogeneity is observed among the patients of beta thalassaemia and sickle cell anaemia due to altered foetal Haemoglobin levels in erythrocytes [1]. Foetal blood comprises of foetal Haemoglobin, that is 70% of total Haemoglobin during birth. Foetal Haemoglobin (HbF) is a mixture of two molecular species i.e., $\alpha_2G\gamma_2$ and $\alpha_2A\gamma_2$. The globin ratio of these molecules is 70:30 in non-affected newborns, whereas in adults, it is 40:60 because of genetic switch [2].

The process of switching of HbA (adult Haemoglobin) to HbF represents important remedial implication in patients suffering from beta thalassaemia. Hydroxyurea is used to increase the level of HbF in patients with beta thalassaemia, but it is toxic in nature. The other drawback associated with hydroxyurea is that it augments the γ globin gene reactivation moderately. Therefore, it is necessary to find out agents having good properties and which could induce higher HbF levels in individuals [3].

Though gene therapy and stem cell transplantation therapies possess potential to treat or cure beta thalassaemia, neither of them is currently applicable to patients of beta thalassaemia, because of the high cost of treatment, varied patients' response, technological issues and absence of sophisticated medical care, which are required to supply these therapies in areas where patients reside. In spite of the advancements in iron chelating therapy, the life expectancies of beta thalassaemia patients have not achieved great success rates. No drugs are known to be safe or effective for the treatment of beta-thalassaemia [4].

Many agents have been depicted, that boost up γ globin gene expression as well as HbF levels in human erythroid culture or in humans, but majority of agents are cytotoxic in nature, damage DNA and have other lethal effects. Hydroxyurea, which is the only approved metabolite used in HbF induction, has been found to be less effective. Natural products like resveratrol and rapamycin are devoid of cytotoxicity or growth inhibitory activity. Theses agents elevate HbF levels in human erythroid precursor cells [4,5].

This review has mainly dealt with the basics of foetal Haemoglobin (HbF) induction and the compounds used for HbF induction.

Genetics of Haemoglobin Switching

Haemoglobin is a tetramer containing two different polypeptide chains i.e., alpha and beta polypeptide chains [6]. The alpha globin chains are encoded by alpha globin genes which are found in duplication and are confined in the telomeric region on the short arm of chromosome 16. The length of the cluster of alpha globin genes is 26 kB and it includes embryonic zeta (ζ) gene, adult alpha1 and alpha2 genes [7,8]. Globin genes encoding beta globin chains are localized on chromosome 11 and they contain embryonic, foetal (Gγ, Aγ), adult δ and β genes [9,10]. Combination of different globin chains results in the formation of a functional Haemoglobin molecule.

The composition of Haemoglobin molecule varies during the gestation and developmental stages of an embryo. During the early stages of gestation, erythropoiesis takes place in the yolk sac. After the first trimester of gestation, first switch accompanied in beta globin cluster, results in transitioning of the process of erythropoiesis, which has to take place in foetal liver. As the time of birth approaches, the outcome of second switch is shifting of erythropoiesis to bone marrow. HbF ($\alpha_2\gamma_2$) is the main Haemoglobin over the phase of gestation and it is progressively substituted by HbA ($\alpha_2\beta_2$) at the stage of infancy [10].

Improper Haemoglobin switch results in higher expression of foetal Haemoglobin, with effective erythropoiesis. This condition is called Hereditary Persistence of Foetal Haemoglobin (HPFH). It has been observed that patients suffering from sickle cell disease and beta thalassaemia, having an improper Haemoglobin switch in their genes, lead normal and healthy lives. So, it can be concluded that induction of foetal Haemoglobin level in thalassaemia patients will help in ameliorate the severities of sickle cell anaemia and beta thalassaemia [11].

Natural Inducers of Haemoglobin F Synthesis

Literature review has stated that the potential use of medicinal plant extracts, mention curative purposes, have been published together with sanative strategies, for restoring healthy state in a number of diseases. Dyslipidaemia, atherosclerosis, hepatitis, bacterial and virus infections are the names of few of such common diseases [12].

In case of beta thalassaemia, few numbers of products from natural world have capability to induce foetal Haemoglobin and to alleviate the pain of disease. Data stating sources and biological effects of these natural compounds have been listed in [Table/Fig-1].

Inducer	Source	Concentration range	Percentage HbF increase (HPLC analysis)	References
Mithramycin	Streptomyces griseus	10 to 20 nM	7.4±1.8	[13,14]
Rapamycin	Streptomyces hygroscopicus	10 to 200 nM	10.2±1.5	[15,16]
Bergapten	Citrus bergamia Risso	400μM	2.31±0.4	[17]
Ethanol extracts of Fructus trichosanthis	Trichosanthes Kirilowii MAXIM	20–80 μg/mL	A 2.6 fold HbF was noted in respect to untreated cells	[18]
Angelicin	Angelica arcangelica	200 to 400 μM	11.2±3.8	[19,20]
Wheatgrass	Triticum aestivum Linn	100 ml	The juice of wheat grass was administered and 3-5 folds HbF augmentation were observed	[21,22]
Resveratrol	Peanuts, Soy beans, Pomegranate, Grape skin	50 μmol/L	A 2.6 fold HbF increase was observed in comparison to untreated cells	[13,23,24]

[Table/Fig-1]: Fetal Haemoglobin inducers from various natural sources

Piceatannol

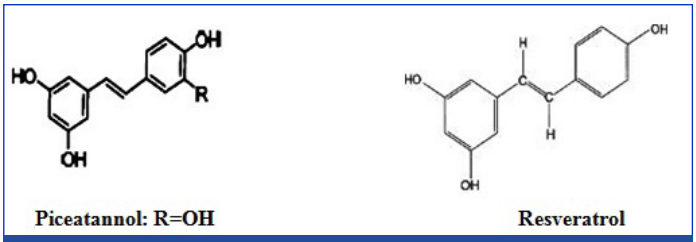
Piceatannol, known as 3, 3', 4', 5-transtrihydroxystilbene, is a powder which has an off white colour. It is not soluble in water but it is soluble in ethanol and dimethyl sulphoxide. Piceatannol contains two phenolic rings which are joined together by a styrene double bond. Cis and trans are the two isomeric forms of piceatannol. Trans isomeric form is sterically more stable in comparison to cis isomeric form [25]. The presence of piceatannol has been confirmed in different fruits and plants such as Vaccinium berries, Vitis amurensi, grapes, Arachis hypogaea, Polygonum cuspidatum [26-29]. The concentration of this compound was found to vary in different sources and it was characterized by different analytical techniques.

Piceatannol possess various health benefits. It possesses anti-adipogenic property, due to which it can modulate the development of adipose tissue [30]. It may be helpful in stimulating the osteoblastic activity, leading to the formation of bone [31]. It is also known to possess antimelanogenic activity [32]. It may also prevent Type 2 diabetes [33].

Piceatannol: Similarity With Naturally Occurring Resveratrol

Piceatannol is a natural polyphenol and a structural analogue of resveratrol [34]. It contains one extra-hydroxyl group in its structure [35]. Synthesis of both the compounds takes place in plants against fungal or in environmentally stressed conditions. Therefore, these are classified as phytoalexins [34]. Chemical structures of both the compounds have been shown below [Table/Fig-2].

[Table/Fig-2]. Chemical structures of piceatannol and resveratrol [36,37]. Both, piceatannol and resveratrol share almost similar properties. Few of these similar properties have been mentioned below [Table/Fig-3].



[Table/Fig-2]: Chemical structures of piceatannol and resveratrol

Resveratrol	Piceatannol	References
✓ Anti- inflammatory activity	✓ Anti- inflammatory activity	[25,38]
✓ Anti-oxidant activity	✓ Anti-oxidant activity	[38,39]
✓ Anti-cancer activity	✓ Anti-cancer activity	[40]
✓ Anti-leukaemic activity	✓ Anti-leukaemic activity	[41]

[Table/Fig-3]: Comparison of similar properties of resveratrol and piceatannol

Data mentioned in literature and research work have stated that piceatannol is a better Anti-oxidant than resveratrol. It has been proved that piceatannol has better activities than resveratrol. Piceatannol is required in very less amounts as compared to resveratrol, for testing of its biological activities [25].

Though evidence shows that resveratrol plays an important role in the prevention of chronic diseases, its meagre bioavailability and fast metabolism limits its use in the prevention of chronic diseases. Analogues of resveratrol, such as piceatannol, overcome the problem of meagre bioavailability and fast metabolism. Therefore, it is more beneficial than resveratrol for improving health and related benefits [42].

Role of p38 Mapk and Erk Pathways in Foetal Haemoglobin Induction

Most of the well described signal pathways in mammals are mitogen-activated protein kinase pathways. They include different subtypes: c-Jun N terminal protein kinase (JNK), p38 and Extracellular Signal Regulated Kinase (ERK). Mitogen activated protein kinases activate each other by phosphorylation in the cytoplasm of the cell and this results in the activation of kinases and various transcription factors. The p38 and JNK pathways are connected to the mechanism of apoptosis in cells and ERK pathway is associated with proliferation and differentiation in cells [38].

The level of activation of mitogen activated protein kinases was observed after the treatment of cells with different concentration of piceatannol. It was observed that the expression of p38 mitogen activated protein kinase was increased and that phospho-ERK expression was decreased in cells treated with piceatannol for 24 hours. Observations of both the pathways were made by comparing the treated cells with control cells (cells not treated with piceatannol) [43].

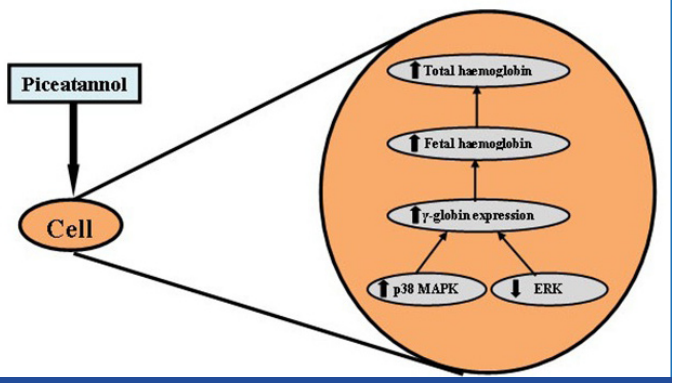
Piceatannol has shown strong Anti-oxidant activity and it has been observed that piceatannol treatment increased the concentration of intracellular Ca_2+ ions and reactive oxygen species, which are known to be the responsible factors of p38 mitogen activated protein kinase activation and ERK inactivation [43].

The mechanism of action of piceatannol has been hypothetically

stated in [Table/Fig-4]. Natural stilbene piceatannol is applied on cultured cells, as shown in [Table/Fig-4]. A magnified view of the cell has been displayed beside that of a treated cell, which shows sequences of events that take place in treated cells.

[Table/Fig-4] A hypothetical illustration of action of piceatannol on mitogen activated protein kinase and that of extra-cellular signal regulated kinase on induction of foetal Haemoglobin. After piceatannol treatment, p38 Mitogen activated protein kinase activation and extra-cellular signal regulated kinase deactivation result in foetal Haemoglobin production and boosting of total Haemoglobin level in U937 cells.

Earlier studies have confirmed that the application of piceatannol



[Table/Fig-4]: Comparison of similar properties of resveratrol and piceatannol

on cells increased the expression of p38 Mitogen Activated Protein Kinase (MAPK) and that it inhibited Extra-cellular Signal Regulated Kinase (ERK) [43]. The expected outcome of these pathways may be the highest expression of gamma globin genes in cells. Because of this, there will be more production of foetal Haemoglobin and an eventual increase in total Haemoglobin levels in cells.

Assumption, Based on Previous Studies

Till date, various reviews, literatures and research works have been published, which have stated the mechanism of action of different naturally occurring compounds and their involvement in reactivation of HbF in patients of beta thalassaemia. All the natural compounds, when they were tested on cells, showed the activation of p38 MAPK (mitogen activated protein kinase) pathway and deactivation of ERK (extra-cellular signal regulated kinase) pathway. It was observed that activation of p38 MAPK (mitogen activated protein kinase) pathway and deactivation of ERK (extra-cellular signal regulated kinase) pathway led to an increase in higher expression of gamma globin gene and an increase in the level of HbF in treated cells.

Increase in gamma globin gene expression and level of foetal Haemoglobin by activation of p38 MAPK pathway and deactivation of ERK pathway, has been observed in cells treated with different naturally occurring pharmacological and chemical compounds, such as cucurbitacin D, hydroxy urea, butyrate, an ethanol extract of Fructus trichosanthis, resveratrol [18,24,44,45].

On the basis of available literature, it may be concluded piceatannol treatment may increase the expression of gamma globin gene and result in increase in foetal Haemoglobin production in beta thalassaemia patients, because activation of p38 MAPK pathway and deactivation of ERK pathway have been confirmed in piceatannol treated cells [46].

CONCLUSION

Augmentation of level of foetal Haemoglobin in patients suffering from beta thalassaemia is an alternative and a promising approach for curing the disease. Various compounds from the natural world and medicinal plants have been identified, which possess the capacity to augment the foetal Haemoglobin levels. Piceatannol, a natural stilbene, possesses a broad range of biological activities which are comparable and similar to activities possessed by compound, resveratrol. Although it is an analogue of resveratrol, it is a less studied compound and its semiquinone radical has a greater stability.

It can be concluded that possession of wide varieties of pharmacological properties, such as Anti-oxidant activity, anti inflammatory activity and anti-tumour activity, strengthen the use of this compound as a pharmacological molecule. However, there is a need to generate further data on the bioavailability and toxicity of piceatannol in humans. Research work needs to be done and data on its capacity to increase foetal Haemoglobin levels and effective doses required to augment the levels of foetal Haemoglobin, needs to be generated.

REFERENCES

[1] Fathallah H, Atweh GF. Induction of fetal hemoglobin in the treatment of sickle cell disease. *Heamatology Am Soc Heamatol Educ Program*. 2006:58-62.
[2] Manca L, Masala B. Disorders of the synthesis of human fetal hemoglobin. *IUBMB Life*. 2008; 60(2):94-111.
[3] Gabbianelli M, Testa U. Role of stem cell factor in the reactivation of human fetal hemoglobin. *Mediterr J Heamatol Infect Dis*. 2009; 1(1):e2009009.
[4] Macari ER, Lowrey CH. Induction of human fetal hemoglobin via the NRF2 Antioxidant response signaling pathway. *Blood*. 2011;117(22):5987-97.
[5] Thein SL. The emerging role of fetal hemoglobin induction in non-transfusion-dependent thalassemia. *Blood Rev*. 2012; 26 (Suppl 1):S35-9.
[6] Sankaran VG, Xu J, Orkin SH. Advances in the understanding of Haemoglobin switching. *Br J Haematol*. 2010;149(2):181-94.
[7] Voon HP, Vadolas J. Controlling alpha-globin: a review of alpha-globin expression and its impact on beta-thalassemia. *Haematologica*. 2008; 93(12):1868-76.
[8] Galanello R, Cao A. Gene test review. Alpha-thalassemia. *Genet Med*. 2011; 13(2):83-8.
[9] Galanello R, Origa R. Beta-thalassemia. *Orphanet J Rare Dis*. 2010; 5:11.
[10] Bauer DE, Kamran SC, Orkin SH. Reawakening fetal hemoglobin: prospects for new therapies for the β-globin disorders. *Blood*. 2012; 120(15):2945-53.
[11] Costa FC, Fedosyuk H, Neades R,1 Bravo de Los Rios J, Barbas CF, Peterson KR. Induction of Fetal Hemoglobin In Vivo Mediated by a Synthetic γ-Globin Zinc Finger Activator. *Anaemia*. 2012; 2012: Article ID 507894.
[12] Bianchi N, Zuccato C, Lampronti I, Borgatti M, Gambari R. Fetal Hemoglobin Inducers from the Natural World: A Novel Approach for Identification of Drugs for the Treatment of β-Thalassemia and Sickle-Cell Anemia. *Evid Based Complement Alternat Med*. 2009; 6(2): 141–51.
[13] Kumari S, Mishra A, Tiwari A. Beta-thalassemia Treatment Passing Through Natural Fetal Heamoglobin Inducers. *Journal of Pharmacy Research*. 2011; 4(10):3851.
[14] Fibach E, Bianchi N, Borgatti M, Prus E, Gambari R. Mithramycin induces fetal hemoglobin production in normal and thalassemic human erythroid precursor cells. *Blood*. 2003; 102(4):1276-81.
[15] Saunders RN, Metcalfe MS, Nicholson ML. Rapamycin in transplantation: a review of the evidence. *Kidney Int*. 2001 Jan;59(1):3-16.
[16] Mischiati C, Sereni A, Lampronti I, Bianchi N, Borgatti M, Prus E, et al. Rapamycin-mediated induction of gamma-globin mRNA accumulation in human erythroid cells. *Br J Haematol*. 2004; 126(4):612-21.
[17] Li H, Ko CH, Tsang SY, Leung PC, Fung MC, Fung KP. The Ethanol Extract of Fructus trichosanthis Promotes Fetal Hemoglobin Production via p38 MAPK Activation and ERK Inactivation in K562 Cells. *Evidence-Based Complementary and Alternative Medicine*. 2011; 2011: Article ID 657056.
[18] El-Beshlawy A, Hamdy M, El Ghamrawy M. Fetal globin induction in beta-thalassemia. *Heamoglobin*. 2009;33 (Suppl 1):S197-203.
[19] Lampronti I, Bianchi N, Borgatti M, Fibach E, Prus E, Gambari R. Accumulation of gamma-globin mRNA in human erythroid cells treated with angelicin. *Eur J Haematol*. 2003; 71(3):189-95.
[20] Swati Padalia, Sushma Drabu, Indira Raheja, Alka Gupta, Mamta Dhamija. Multitude potential of wheatgrass juice (Green Blood): An overview. *J Postgrad Med*. 2010; 1(3):23-28.
[21] Singh LK, Rai M, Chaudhary M. Intellectual Properties Rights-A strong determinant of economic growth in agriculture. *Chronicles of Young Scientists*. 2010; 1(2): 29-34.
[22] Rana S, Kamboj JK, Gandhi V. Living life the natural way–Wheatgrass and Health. *Functional Food in Health and Disease*. 2011; 11: 444-56.
[23] Catalgol B, Batirel S, Taga Y, Ozer NK. Resveratrol: French paradox revisited. *Front Pharmacol*. 2012; 3:141.
[24] Rodrigue CM, Arous N, Bachir D, Smith-Ravin J, Romeo PH, Galacteros F, Garel MC. Resveratrol, a natural dietary phytoalexin, possesses similar properties to hydroxyurea towards erythroid differentiation. *Br J Haematol*. 2001; 113(2):500-7.
[25] Piotrowska H, Kucinska M, Murias M. Biological activity of piceatannol: leaving the shadow of resveratrol. *Mutat Res*. 2012; 750(1):60-82.
[26] Rimando AM, Kalt W, Magee JB, Dewey J, Ballington JR. Resveratrol, pterostilbene, and piceatannol in vaccinium berries. *J Agric Food Chem*. 2004 ; 52(15):4713-9.

[27] Ha do T, Chen QC, Hung TM, Youn UJ, Ngoc TM, Thuong PT, et al. Stilbenes and oligostilbenes from leaf and stem of Vitis amurensis and their cytotoxic activity. *Arch Pharm Res*. 2009 ; 32(2):177-83.
[28] Bavaresco L, Fregoni MARIO, Trevisan Mattivi F, Vrhovsek U, Falchetti R. The occurrence of the stilbene piceatannol in grapes. *Vitis*. 2002; 41: 133-36.
[29] Lin LL, Lien CY, Cheng YC, Ku KL. An effective sample preparation approach for screening the Anti-cancer compound piceatannol using HPLC coupled with UV and fluorescence detection. *J Chromatogr B Analyt Technol Biomed Life Sci*. 2007; 853(1-2):175-82.
[30] Kwon JY, Seo SG, Heo YS, Yue S, Cheng JX, Lee KW, Kim KH. Piceatannol, Natural Polyphenolic Stilbene, Inhibits Adipogenesis via Modulation of Mitotic Clonal Expansion and Insulin Receptor-dependent Insulin Signaling in Early Phase of Differentiation. *The Journal of Biological Chemistry*. 287 (14): 11566–78.
[31] Chang JK, Hsu YL, Teng IC, Kuo PL. Piceatannol stimulates osteoblast differentiation that may be mediated by increased bone morphogenetic protein-2 production. *Eur J Pharmacol*. 2006;551(1-3):1-9.
[32] Matsui Y, Sugiyama K, Kamei M, Takahashi T, Suzuki T, Katagata Y, et al. Extract of passion fruit (Passiflora edulis) seed containing high amounts of piceatannol inhibits melanogenesis and promotes collagen synthesis. *J Agric Food Chem*. 2010; 58: 11112–18.
[33] Minakawa M, Miura Y, Yagasaki K. Piceatannol, a resveratrol derivative, promotes glucose uptake through glucose transporter 4 translocation to plasma membrane in L6 myocytes and suppresses blood glucose levels in type 2 diabetic model db/db mice. *Biochem Biophys Res Commun*. 2012; 422(3):469-75.
[34] Wolter F, Clausnitzer A, Akoglu B, Stein J Piceatannol. A natural analog of resveratrol, inhibits progression through the S phase of the cell cycle in colorectal cancer cell lines. *J Nutr*. 2002; 132(2):298-302.
[35] Potter GA, Patterson LH, Wanogho E, Perry PJ, Butler PC, Ijaz T, Ruparelia KC, Lamb JH, Farmer PB, Stanley LA, Burke MD. The cancer preventative agent resveratrol is converted to the Anti-cancer agent piceatannol by the cytochrome P450 enzyme CYP1B1. *Br J Cancer*. 2002; 86(5):774-8.
[36] Okuda T, Ito H. Tannins of Constant Structure in Medicinal and Food Plants-Hydrolyzable Tannins and Polyphenols Related to Tannins. *Molecules*. 2011; 16(3): 2191-2217.
[37] Marques FZ, Markus MA, Morris BJ. Resveratrol: cellular actions of a potent natural chemical that confers a diversity of health benefits. *Int J Biochem Cell Biol*. 2009; 41(11):2125-8.
[38] King RE, Bomser JA, Min DB. Bioactivity of resveratrol. *Comprehensive Reviews in Food Science and Food Safety*. 2006; 5: 65-70.
[39] Kim HJ, Lee KW, Lee HJ. Protective effects of piceatannol against beta-amyloid-induced neuronal cell death. *Ann N Y Acad Sci*. 2007; 1095:473-82.
[40] Wesołowska O, Kuzdzał M, Strancar J, Michalak K. Interaction of the chemopreventive agent resveratrol and its metabolite, piceatannol, with model membranes. *Biochim Biophys Acta*. 2009; 1788(9):1851-60.
[41] Wieder T, Prokop A, Bagci B, Essmann F, Bernicke D, Schulze-Osthoff K, et al. Piceatannol, a hydroxylated analog of the chemopreventive agent resveratrol, is a potent inducer of apoptosis in the lymphoma cell line BJAB and in primary, leukaemic lymphoblasts. *Leukaemia*. 2001; 15(11):1735-42.
[42] Minakawa M, Miura Y, Yagasaki K. Piceatannol, a resveratrol derivative, promotes glucose uptake through glucose transporter 4 translocation to plasma membrane in L6 myocytes and suppresses blood glucose levels in type 2 diabetic model db/db mice. *Biochem Biophys Res Commun*. 2012;422(3):469-75.
[43] Liu WH, Chang LS. Piceatannol induces Fas and FasL up-regulation in human leukemia U937 cells via Ca2+/p38alpha MAPK-mediated activation of c-Jun and ATF-2 pathways. *Int J Biochem Cell Biol*. 2010 Sep; 42(9):1498-506.
[44] Liu K, Xing H, Zhang S, Liu Sm, Fung Mc. Cucurbitacin D, induces fetal hemoglobin synthesis in K562 cells and human hematopoietic progenitors through activation of p38 pathway and stabilization of the γ-globin mRNA. *Blood Cells Mol Dis*. 2010; 45(4):269-75.
[45] Pace BS, Zein S. Understanding mechanisms of gamma-globin gene regulation to develop strategies for pharmacological fetal hemoglobin induction. *Dev Dyn*. 2006; 235(7):1727-37.
[46] Liu WH, Chang LS. Suppression of Akt/Foxp3-mediated miR-183 expression blocks Sp1-mediated ADAM17 expression and TNFα-mediated NFκB activation in piceatannol-treated human leukemia U937 cells. *Biochem Pharmacol*. 2012; 84(5):670-80.

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