The Efficacy of Topical Vitexin on Hair Growth Promotion in Mice

Rueangrit Siriphanit[1], Saranyoo Ponnikorn[2], Werayut Yingmema[3], Jitlada Meephansan[*1]

[1]Division of Dermatology, Chulabhorn International College of Medicine, Thammasat University, Pathum Thani, Thailand
[2]Chulabhorn International College of Medicine, Thammasat University, Pathum Thani, Thailand
[3]Laboratory Animal Center, Thammasat University, Pathum Thani, Thailand
[*]Corresponding author, E-mail: kae_mdcu@yahoo.com

Abstract

Vitexin, a naturally occurring polyphenol classified as a flavonoid compound, was previously known as herbal medicine in China with anti-inflammatory, anti-oxidative and anti-cancer activities. Moreover, the recent *in vitro* study showed that vitexin compound-1 (VB-1), which is isolated from vitexin, can augment Wnt/β-catenin signaling in human dermal papilla cells (hDPCs) and significantly promotes the proliferation of hDPCs. In this study, the efficacy of hair growth-promoting effects with topical vitexin was compared with solution vehicle (60% ethanol) in mice model. Twenty-six-week-old male C57BL/6 mice will be clipped on the dorsal skin area and randomly assigned to 2 groups (10 mice/group). Each group will be respectively treated with 0.2% topical vitexin and the solution vehicle alone once daily for 21 days. The digital image of the clipped area was recorded on day 0, 7, 14, and 21. The results demonstrated that vitexin-treated group had hair regrowth and promoted rate of hair growth similar to the control group (P = 0.189, P = 0.534, respectively). Thus, the results conclude that 0.2% of topical vitexin has no efficacy on hair growth promotion.

Keywords**:** *Vitexin, Androgenetic alopecia, Hair growth, Mice model*

1. Introduction

Hair loss (alopecia) is a prevalent dermatological course and has an impact on both females and males of all ages. Androgenetic alopecia (AGA) is one of those and has been known to be the most common type of non-scarring alopecia. One of the key features of AGA is a progressive reduction in the duration of the anagen phase associated with premature catagen phase, resulting in progressive miniaturization of hairs, because ectopic activation of androgen receptor signaling responding to dihydrotestosterone (DHT) in the hair follicle, mainly in the dermal papilla, alters the expression of hair growth-related paracrine factors (Luo et al., 2018; Kwack et al., 2008). Together with the androgen-dependent process, genetic and pathogenic factors, including microbial flora, stress and microinflammation, are involved in the pathogenesis of AGA. In order to focus on the molecular mechanisms, Wnt/β-catenin is known to be the key role in anagen-inducing signal (Greco et al., 2009; Schneider et al., 2009). Central to this process is the stabilization of β-catenin, an established effector of active Wnt signaling and a transcriptional cofactor for LEF1/TCF proteins (Greco et al., 2009). In addition to Wnt/β-catenin, BMP antagonists (such as Noggin) and SHH also act as anagen-inducing signals (Schneider et al., 2009). In order to maintain the anagen phase, IGF-1, HGF, and VEGF are thought to be important (Schneider et al., 2009; Paus & Foitzik; 2004). In contrast, FGF5 is shown to be a key inducer of catagen (Schneider et al., 2009; Paus & Foitzik; 2004). Moreover, TGF-β1, IL-1β, the neurotrophins (NT-3, NT-4 and BDNF), BMP2/4 and TNF-α have been described to induce catagen (Schneider et al., 2009; Paus & Foitzik; 2004). In the same way, activation of BMP signaling together with Wnt inhibition by TCF3 and DKKs inhibits initiation of the anagen phase of Wnt/β-catenin signaling (Schneider et al., 2009). In contrast to its high prevalence and wide influence of AGA, the approved therapeutic options including oral finasteride and topical minoxidil are still limited in efficacy together with adverse events, leading to poor compliance problems. Recently, the discovery of vitexin, a naturally occurring polyphenol classified as a flavonoid compound, has been proposed. It has been traditionally used as a herbal medicine in China for cough, asthma, rheumatism, and arthritis (Zhou et al., 2009; Xin et al., 2013) and shown to possess a variety of pharmacological effects, including anti-inflammatory effects (Borghi et al., 2013; Sun et al., 2016), anti-oxidant effects (Borghi et al., 2013; Sun et al., 2016), antineoplastic effects, protective

effects against neurological and psychiatric diseases, protective activities in the cardiovascular system, protective effects against endocrine and metabolic diseases, anti-microbial effects and anti-viral effects. Moreover, the recent *in vitro* study showed that vitexin compound-1 (VB-1), which is isolated from vitexin, can augment Wnt/β- catenin signaling in human dermal papilla cells (hDPCs) and significantly promotes the proliferation of hDPCs (Luo et al., 2018). So, vitexin could be beneficial in treating non-scarring alopecia because it can provide multiple therapeutic rationales on hair growth-promoting effects which are augmenting Wnt/β-catenin signaling in hDPCs, promoting the proliferation of hDPCs, elongating of hair shafts, anti-oxidative effects and also anti-inflammation effects. However, the mechanisms and efficacy of vitexin are still not completely clear; hence further study should be done. In this study, we attempted to examine the efficacy of promoting hair growth in mice models with topical vitexin. The results will be advantageous in applying to develop a new alternative therapeutic agent for patients who suffered from non-scarring alopecia in the near future.

2. Objectives

To study the effects of 0.2% topical vitexin on hair growth promotion in mice model

3. Materials and Methods

3.1 Animals: Five-week-old C57BL/6 male mice were housed under a strict hygienic conventional standard, maintained under controlled environmental conditions (12:12 hour light and dark cycle, light 130-325 Lux, temperature approximately 22 +/- 1°C, relative humidity 30-70%), and provided with standard laboratory food and water ad libitum for one week. After six weeks, C57BL/6 male mice were randomly assigned to vitexin-treated group (N=10) and control group (N=10). The study protocol was approved by the Animal Ethical Committee at Thammasat University, Thailand.

3.2 Drugs used in the study: Vitexin was purchased from Sigma-Andrich (CAS Number: 3681-93-4) and dissolved in 60% ethanol to prepare 0.2% concentration. The solution vehicle alone (60% ethanol) was used as a control group.

3.3 Study design: The six-week-old male mice were anesthetized with inhaled isoflurane (Attane™, Piramal, USA) one day before the experiment (day 0) to shave an area of hair on the dorsal skin of all mice under sterile conditions. Then, the mice were randomly divided into a group of ten and labeled. On day 1 until day 21, each group was topically treated once daily with 0.2% vitexin or 60% ethanol (control group) 0.1 mL/area. Photographic data was recorded weekly until three weeks using a digital camera (Sony A7 III, Sony Corporation, Japan) and microscope (Dino-Lite AM7013MZT(R4), AnMo Electronics Corporation, Taiwan) at 65x magnification focusing on the dorsal back coated area. Photographic data was analyzed by using Photoshop 6.0 software to calculate the ratio of hair regrowth area to denuded area, and targeted area hair elongation measurement was also analyzed using the Dino-Lite microscope. At experimental endpoint, mice humanely euthanized with the maintenance of inhaled isoflurane (Attane, USA) to the deep stage of anesthesia followed by cervical dislocation.

4. Results and Discussion

4.1 Vitexin-treated group showed comparable hair regrowth compared to the control group

Of the 20 mice treated, all remained healthy. On day 21 after treatment, mice treated with vitexin showed more area of hair regrowth with non-statistical significance on the dorsal coated area (vitexin-treated group (74.49 ± 10.47%) compared to the control group (65.32 ± 18.44%), P-value = 0.189) (Table 1, Figure 1).

Table 1 Comparison of area hair regrowth (%) at days 0, 7, 14, and 21 between vitexin-treated and control groups.

Day	Vitexin (n=10) Mean ± SD	Control (n=10) Mean ± SD	P-value
Day 0	11.33 ± 7.43	8.65 ± 6.66	0.406
Day 7	21.72 ± 5.8	18.19 ± 7.02	0.236
Day 14	69.63 ± 11.28	60.11 ± 20.08	0.212
Day 21	74.49 ± 10.47	65.32 ± 18.44	0.189

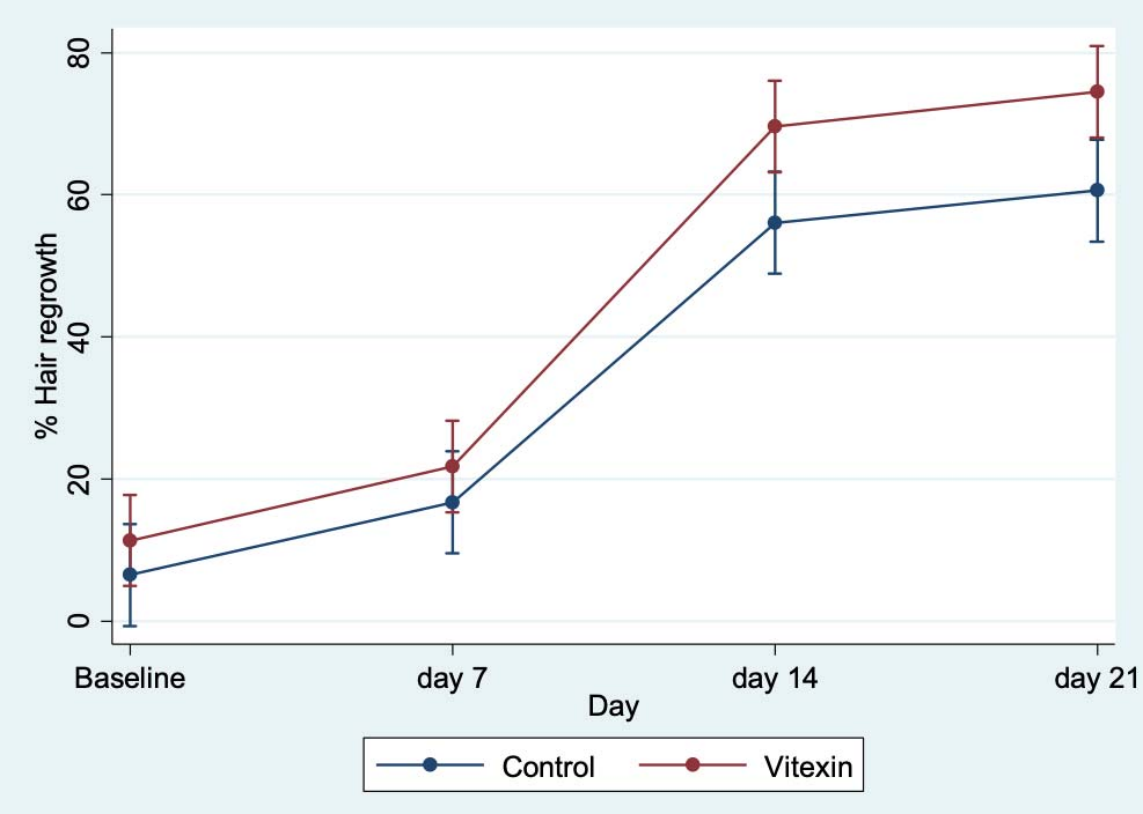

Figure 1 Comparison of area hair regrowth (%) at days 0, 7, 14, and 21 between vitexin-treated and control groups.

From observation, we could grossly see thinned-quality hair regrowth initiated on the dorsal coated area of mice treated with vitexin approximately since day 7 after treatment. Hair regrowth continued up to the end of the study (21 days) with gradually larger in hair diameter and density, as shown in Figure 2.

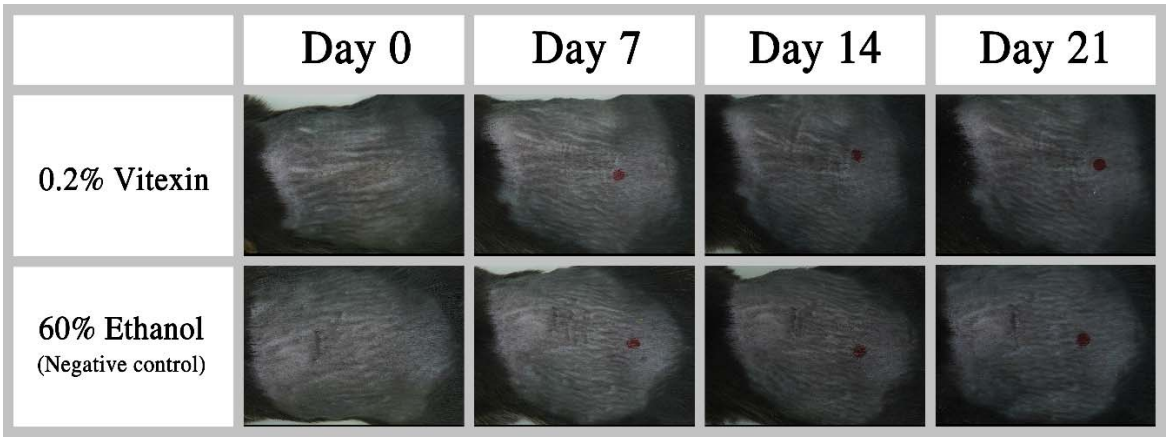

Figure 2 Hair regrowth on days 0, 7, 14, and 21 after topical application of 0.2% vitexin compare with control. During the experiment, hair regrowth gradually continued until the entire study with larger in diameter and density of hair.

4.2 Vitexin-treated group revealed non-significantly rapid rate of hair growth

According to the experiment, the researcher examined hair growth rate calculated from the mean hair length (mm.) which was measured using a digital microscope from day 0 till day 21 after treatment. Then, it was found that the mice treated with topical vitexin had a more non-significantly rapid rate of hair growth as compared to the control at day 14 and 21 (vitexin-treated group (1.19 ± 0.62 and 1.12 ± 0.52 (mm. x 10^{-2}), respectively) compared to the control group (0.87 ± 0.55 and 0.97 ± 0.46 (mm. x 10^{-2}), respectively), P-value = 0.262 and 0.534), respectively) (Table 3).

Table 2 Comparison of length of hair regrowth between vitexin-treated and control group calculated from mean hair length (mm.) from day 0 - 21 of treatment.

Day	Vitexin (n=10) Mean ± SD	Control (n=8) Mean ± SD	P-value
Day 0	0.36 ± 0.06	0.36 ± 0.13	0.955
Day 7	0.46 ± 0.09	0.47 ± 0.15	0.829
Day 14	0.53 ± 0.09	0.48 ± 0.12	0.345
Day 21	0.6 ± 0.07	0.56 ± 0.11	0.452

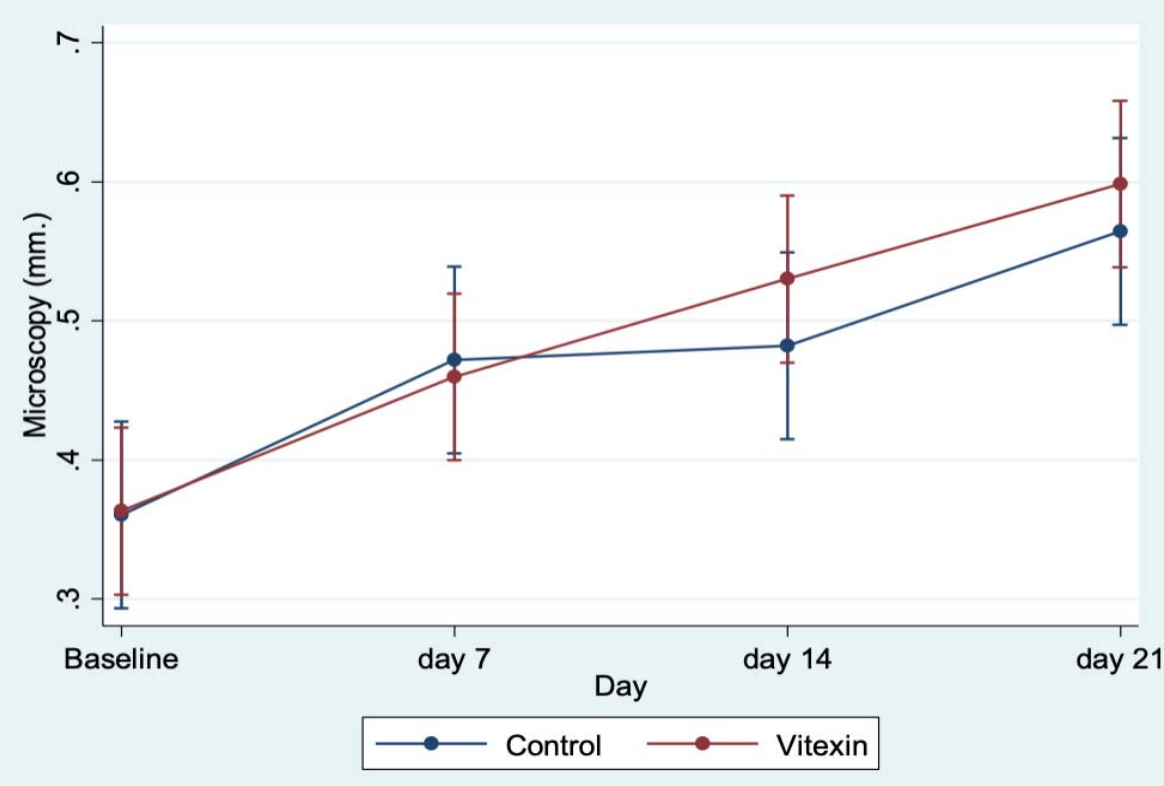

Figure 3 Comparison of length of hair regrowth between vitexin-treated and control group calculated from mean hair length (mm.) from day 0 - 21 of treatment.

Table 3 Comparison of the average rate of hair regrowth between vitexin-treated and control group calculated from mean hair length (mm. x 10^{-2}) from day 0 until day 7, 14, 21 of treatment.

Day	Vitexin (n=10) Mean ± SD	Control (n=8) Mean ± SD	P-value
Day 0-7	1.38 ± 0.9	1.59 ± 1.19	0.668
Day 0-14	1.19 ± 0.62	0.87 ± 0.55	0.262
Day 0-21	1.12 ± 0.52	0.97 ± 0.46	0.534

Corresponding to our clinical data, vitexin could promote hair regrowth, which this ascertains the previous *in vitro* study findings and can be explained as the comprehensive effect of the activation of Wnt/β-catenin signaling in hDPCs (Luo et al., 2018) and upregulation of Wnt signaling-associated signature genes of the dermal papilla, LEF1, Wnt5a, ALPL and VCAN (Luo et al., 2018). In contrast, DKK1 and AXIN2, a negative regulator of Wnt/β-catenin signaling, were down-regulated (Luo et al., 2018). Another feasible mechanism of vitexin that supports the results is its anti-inflammatory effect which could be another additional potential in promoting hair growth by the inhibition process of hair follicle microinflammation, one of important pathogenesis of AGA. It is related to AGA for both clinical signs of perifollicular inflammatory and perifollicular lymphocytic infiltration in scalp biopsy. Vitexin could exhibit anti-inflammatory effects via the inhibition of various inflammatory cytokines, including IL-1β (Borghi et al., 2013; Sun et al., 2016; Rosa et al., 2016), IL-6 (Borghi et al., 2013; Sun et al., 2016; Kang et al., 2015), IL-8 (Flores et al., 2012), IL-17 (Reis et al., 2014), IL-33 (Borghi et al., 2013), TNF-α (Borghi et al., 2013; Sun et al., 2016; Rosa et al., 2016; Reis et al., 2014; Nikfarjam et al., 2017), NF-κB (Sun et al., 2016), NO (Rosa et

al., 2016; Nikfarjam et al., 2017), PGE2 (Rosa et al., 2016)11, MCP-1 (Kang et al., 2015) and neutrophil influx (Rosa et al., 2016; De Melo et al., 2005). Vitexin could also reduce the expression of p-p38, p-ERK and p-JNK (Rosa et al., 2016). Moreover, vitexin could also enhance the anti-inflammatory cytokine, including IL-10 (Borghi et al., 2013). However, in this study, the result of hair growth promotion of vitexin in mice showed more non-significantly hair regrowth compared to the control group. The higher dose of topical vitexin used and the longer duration of application time are believed to be the main factors that could affect the therapeutic outcome to be significant.

5. Conclusion

In conclusion, topical vitexin has non-significantly higher efficacy as a hair growth promoter than control. The author suggests continuing further studies using varied doses of topical vitexin to examine its efficacy and adverse events in an animal model. Besides, further investigations are needed to determine if vitexin could show a significant therapeutic outcome and maintain its significant therapeutic effects on hair growth after treatment cessation, which is a limitation of an approved therapeutic drug for non-scarring alopecia.

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7. References

Borghi, S. M., Carvalho, T. T., Staurengo-Ferrari, L., Hohmann, M. S. N., Pinge-Filho, P., Casagrande, R., & Verri, W. A. (2013). Vitexin Inhibits Inflammatory Pain in Mice by Targeting TRPV1, Oxidative Stress, and Cytokines. *Journal of Natural Products*, *76*(6), 1141–1149. doi: 10.1021/np400222v

Flores, G., Dastmalchi, K., Dabo, A. J., Whalen, K., Pedraza-Peñalosa, P., Foronjy, R. F., … Kennelly, E. J. (2012). Antioxidants of therapeutic relevance in COPD from the neotropical blueberry Anthopterus wardii. *Food Chemistry*, *131*(1), 119–125. doi: 10.1016/j.foodchem.2011.08.044

Greco, V., Chen, T., Rendl, M., Schober, M., Pasolli, H. A., Stokes, N., Fuchs, E. (2009). A Two-Step Mechanism for Stem Cell Activation during Hair Regeneration. *Cell Stem Cell*, *4*(5), 464. doi: 10.1016/j.stem.2009.03.018

Kang, I., Choi, S., Ha, T. J., Choi, M., Wi, H.-R., Lee, B. W., & Lee, M. (2015). Effects of Mung Bean (Vigna radiata L.) Ethanol Extracts Decrease Proinflammatory Cytokine-Induced Lipogenesis in the KK-Ay Diabese Mouse Model. *Journal of Medicinal Food*, *18*(8), 841–849. doi: 10.1089/jmf.2014.3364

Kwack, M. H., Sung, Y. K., Chung, E. J., Im, S. U., Ahn, J. S., Kim, M. K., & Kim, J. C. (2008). Dihydrotestosterone-Inducible Dickkopf 1 from Balding Dermal Papilla Cells Causes Apoptosis in Follicular Keratinocytes. *Journal of Investigative Dermatology*, *128*(2), 262–269. doi: 10.1038/sj.jid.5700999

Luo, J., Chen, M., Liu, Y., Xie, H., Yuan, J., Zhou, Y., Ding, J., Deng, Z. & Li, J. (2018). Nature-derived lignan compound VB-1 exerts hair growth-promoting effects by augmenting Wnt/β-catenin signaling in human dermal papilla cells. *PeerJ*, *6*. doi: 10.7717/peerj.4737

Melo, G. O. D., Muzitano, M. F., Legora-Machado, A., Almeida, T. A., Oliveira, D. B. D., Kaiser, C. R., Costa, S. S. (2005). C-Glycosylflavones from the Aerial Parts ofEleusine indicaInhibit LPS-Induced Mouse Lung Inflammation. *Planta Medica*, *71*(4), 362–363. doi: 10.1055/s-2005-864104

Nikfarjam, B. A., Hajiali, F., Adineh, M., & Nassiri-Asl, M. (2017). Anti-inflammatory Effects of Quercetin and Vitexin on Activated Human Peripheral Blood Neutrophils:-The effects of quercetin and vitexin on human neutrophils. *Journal of pharmacopuncture*, *20*(2), 127-131.

Paus, R., & Foitzik, K. (2004). In search of the "hair cycle clock": a guided tour. *Differentiation*, *72*(9-10), 489–511. doi: 10.1111/j.1432-0436.2004.07209004.x

Reis, G. O. D., Vicente, G., Carvalho, F. K. D., Heller, M., Micke, G. A., Pizzolatti, M. G., & Fröde, T. S. (2013). Croton antisyphiliticus Mart. attenuates the inflammatory response to carrageenan-induced pleurisy in mice. *Inflammopharmacology*, *22*(2), 115–126. doi: 10.1007/s10787-013-0184-6

Rosa, S. I. G., Rios-Santos, F., Balogun, S. O., & Martins, D. T. D. O. (2016). Vitexin reduces neutrophil migration to inflammatory focus by down-regulating pro-inflammatory mediators via inhibition of p38, ERK1/2 and JNK pathway. *Phytomedicine*, *23*(1), 9–17. doi: 10.1016/j.phymed.2015.11.003

Schneider, M. R., Schmidt-Ullrich, R., & Paus, R. (2009). The Hair Follicle as a Dynamic Miniorgan. *Current Biology*, *19*(3). doi: 10.1016/j.cub.2008.12.005

Sun, Z., Yan, B., Yu, W. Y., Yao, X., Ma, X., Sheng, G., & Ma, Q. (2016). Vitexin attenuates acute doxorubicin cardiotoxicity in rats via the suppression of oxidative stress, inflammation and apoptosis and the activation of FOXO3a. *Experimental and Therapeutic Medicine*, *12*(3), 1879–1884. doi: 10.3892/etm.2016.3518

Xin, H., Kong, Y., Wang, Y., Zhou, Y., Zhu, Y., Li, D., & Tan, W. (2013). Lignans extracted from Vitex negundo possess cytotoxic activity by G2/M phase cell cycle arrest and apoptosis induction. *Phytomedicine*, *20*(7), 640–647. doi: 10.1016/j.phymed.2013.02.002

Zhou, Y., Liu, Y. E., Cao, J., Zeng, G., Shen, C., Li, Y., Shi, Y. E. (2009). Vitexins, Nature-Derived Lignan Compounds, Induce Apoptosis and Suppress Tumor Growth. *Clinical Cancer Research*, *15*(16), 5161–5169. doi: 10.1158/1078-0432.ccr-09-0661