行政院國家科學委員會專題研究計畫 期末報告

探討銀杏葉萃取物及其相關活性成份對傷害性光線誘發小鼠眼組織損傷退化之保護效果

計 畫 類 別 ：個別型
計 畫 編 號 ：NSC 101-2320-B-040-012-
執 行 期 間 ：101 年 08 月 01 日至 102 年 07 月 31 日
執 行 單 位 ：中山醫學大學視光學系

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公 開 資 訊 ：本計畫涉及專利或其他智慧財產權，2 年後可公開查詢

中 華 民 國 102 年 10 月 31 日

中 文 摘 要 ： 1 紫外線（UV）過量接觸會造成眼球表面損傷及退化，並誘發多種發炎反應，本研究以動物實驗方法探討銀杏葉萃取物（Ginkgo Biloba Leaf Extract, Gk）對紫外線 B（UVB）輻射誘導眼表組織光毒性的預防作用，並討論機轉及其功能性成份的保健效果。實驗組別分為：未做任何處理的 Blank Control 組，UVB 組以及分別經口投藥銀杏葉萃取物（Gk）的組別。視覺的評估模式採取角膜平滑度評估方法、角膜地形圖評估方法、不透明度程度評估方法和眼表損傷染色程度評估方法。另採取組織進行 H&E 染色（Hematoxylin and Eosin stain）與結膜杯狀細胞 PAS 染色（Periodic acid-Schiff stain）。動物結果發現，銀杏葉萃取物與（Gk）2 mg/kg、10 mg/kg 的作用下，可預防 UVB 誘導角膜損傷退化。組織病理分析發現，銀杏葉萃取物（Gk）是透過抑制 UVB 誘發的發炎機轉，結果發現銀杏葉萃取物（Gk）可阻斷 UVB 誘導眼角膜組織表現 NF-κB、COX-2、Fas 和 MMP-9 蛋白。UVB 會造成結膜杯狀細胞的顆數與飽和度下降，於銀杏葉萃取物（Gk）10 mg/kg 的作用下，可使結膜杯狀細胞維持較好的狀況。綜上所述，銀杏葉萃取物（Gk）於動物實驗發現減緩及預防 UVB 誘導角膜與結膜損傷。本研究合理地推論，銀杏葉萃取物（Gk）具有潛力開發成預防或治療因過度的紫外線照射的眼表傷害。本研究「動物視覺健康實驗評估技術模式」具有相當的社會經濟發展價值，探討此計畫研究目的同時，額外建立此動物模式用以驗證認證「護眼健康食品」的效益，有助於未來政府持續推動「健康食品的認證」政策。

中文關鍵詞： 銀杏葉萃取物、護眼建康食品、護眼健康食品認證模式開發、退化性視覺疾病、角膜、結膜

英 文 摘 要 ： Purpose: Ultraviolet B (UVB) irradiation activates nuclear factor-kappa B (NF-κB), COX-2 , Fas and MMP-9 in the cornea, resulting in inflammatory responses and polymorphonuclear leukocytes (PMN) accumulation in the corneal stroma. This study aims to determine the effect of ginkgo biloba leaf extract (Gk), a potent ocular surfaces inflammation modulators, on UVB-induced corneal damages in a mouse model.

Methods: ICR mice were randomly divided into five groups. The mice were anaesthetized with their ocular surfaces exposed to UVB light (0.72J/cm2/daily), followed by daily treatment with ginkgo biloba leaf extract (Gk) at 0 mg/kg, 0.4 mg/kg, 2 mg/kg and 10

mg/kg of body weight. Mice without Gk supplements were used as treatment controls and mice without UVB irradiation as blank controls. Corneal surface damages were graded according to smoothness, opacity, and the extent of lissamine green staining. Histopathological changes were also examined, along with the expression of NF-κB-p65, COX-2, and MMP-9. PMN accumulation and the expression of CK-5, P63 and Fas protein were also examined.

Results: UVB irradiation caused significant damages to cornea, including sustained inflammation, apparent corneal ulcer, and severe epithelial exfoliation, leading to thinning of corneal epithelial layer and infiltration of polymorphonuclear leukocytes (PMN). NF-κB-p65 expression was highly activated with nuclear translocation. The expression of COX-2 and Fas were increased. MMP-9 positive cells accumulation was also increased in the stroma. With dietary ginkgo biloba leaf extract (Gk), corneal damages were ameliorated in a dose-dependent manner. NF-κB-p65 activation and its nuclear translocation were blocked with decreased expression of COX-2 and Fas. Infiltration of polymorphonuclear leukocytes (PMN) was also blocked by dietary ginkgo biloba leaf extract prior to UVB irradiation. Besides, MMP-9 positive cells accumulation was reduced in the stroma with concomitant in a dose-dependent manner.

Conclusions: Results of this study suggest that ginkgo biloba leaf extract (Gk) may be used as a prophylactic agent against UVB-induced photokeratitis.

英文關鍵詞： Ginkgo Biloba extract, vision protection, vision degenerative diseases

(一) 研究背景與研究目標：

◎ 視覺的產生必須依賴光的傳導，視覺的產生過程，需透過光與神經系統的整合與協調轉換，從遠距離(大腦迴路)的控制眼球內的睫狀肌「調節力」以及眼肌「聚合力」，協同瞳孔的調節反應，將影像正確的投射在視網膜上，幫助我們產生清楚的視覺。然而，照明光源又是近代造成視覺不健康的主要元凶之一。

◎ 照明的最終使用者是人類，這就涉及到光的質量，尤其是對人類眼球組織和視覺的安全是有所疑慮的。隨著智慧型手機與平板電腦的普及(LED 背光照明)，威脅人類視覺健康的風險因子，已由典型的近視、散光、老花的問題，逐漸轉移成視網膜功能早衰和老化、增加病變的風險。對於 LED 之「光生物安全」及其對「人眼及視覺的安全性問題」正在引起國際國內各方面的關注，或許正因如此，開發「減緩」或「預防」它對視覺的慢性傷害的方法就顯得更重要 [1]。近代國人視覺健康逐年下降，視覺退化疾病有逐年增加和年輕化的趨勢，其中又以**角結膜**或**視網膜的退化**最為常見。其問題可歸咎於現代生活中的傷害性輻射光源，如：**紫外線**。雖然人的眼睛感測不到這些傷害性輻射光源，但已有許多研究報告指出，長期接觸會此傷害性的光源與視覺組織的損傷和視力退化有密切的關係 [2, 3, 4 ,5]。因過去採取傳統光學鏡片阻隔傷害性光輻射的效果不佳，並且視覺退化問題一旦發現時，多半視力已嚴重的降低，即使接受了最先進治療，視覺品質重建或視力恢復能力不理想。尋找更好的「預防保健」方法，為早期預防以及降緩視覺快速退化的方法提供了新策略。銀杏葉萃取物(Ginkgo biloba extract)為本計劃研究的選擇。

◎ 當代LED系統的設計發展，常注重在追求高色溫、高輝度、省電、高閃頻、高演色性等技術及節能層面，但往往忽略了所研發出來的照明設備，真正對人眼的影響？未來隨著LED光效、亮度的提高，尤其是隨著LED的衍生應用越來越廣泛，例如：電視、電腦、智慧型手機、燈泡、檯燈。然而，一旦LED大規模進入室內照明，它的安全性就顯得更重要。或許正因如此，人類眼球視覺系統暴露於**紫外線**和**LED照明系統**所產生的「光衝擊傷害」的機會越來越高。目前少有研究著重在LED光源於生物視覺安全性評估，LED 之「光生物安全」之設計標準亦尚未被明確的規範出來。正因如此，現代人視覺健康的風險相對的高出許多，更需要積極的尋求防護的方法。臨床上，視覺退化問題與傷害一旦發現時，多半視力已嚴重的降低，即使接受了最先進治療，視覺品質重建或視力恢復的結果並不理想，況且目前乃無特效藥。因此尋找更好的預防保健方法迫在眉睫，尤其當代眼視覺相關之「生技健康食品」概念的興起，為早期預防或於降緩視覺快速退化的策略提供了一種新思路；此外，若能開發出可「**反轉**」輕度視網膜損害病人的視覺品質之方法，將更有幫助於眼疾病的患者。

◎ 在醫藥及建康食品領域，目前對於銀杏葉萃取物(Ginkgo biloba extract)用於眼球表面保健功效、視覺功能性分析和學理證據還不夠完整，事實上有許多潛在性的護眼功效需進一步整體性的分析與評估。透由此計畫的執行，可清楚的了解**銀杏葉萃取物(Ginkgo biloba extract)對於預防眼球表面受紫外線傷害的能力**，以實驗動物方法「認證」銀杏葉萃取物及其相關活性成份之護眼功效。本研究的產出，將有助於醫療生技產業或傳統中草藥保健醫學的發展。以下的護眼保健的課題，由本研究的成果可得初步的解答。銀杏葉萃取物預防紫外線角膜炎的能力與減緩紫外線誘導角膜

表現NF-κB、COX-2 和MMP-9 蛋白的表現。

◎ 本研究以「生物視覺模式認證」的方法，探討「食用天然活性成份」對於「護眼保健的功效」與「視覺功能性生理評估」，目前已有相當的經驗與成效。同時，102 年度研究計劃延續本期國科會計畫，持續發展保健中草藥-銀杏葉萃取物的基礎研究與應用，探討銀杏葉萃取物發展應用於退化性視覺疾病的防治的可能性，以開發「護眼機能性保健食品」為終極目標。目前台灣，「視覺機能性保健食品」的「生物認證系統」尚未成熟，**本研究計畫另一價值在於藉由光誘導視覺損傷動物模式(紫外線與L)，建立可用於分析與認證護眼功能性天然成份的評估系統。**

(a) 紅球薑萃取物 (Zerumbone, ZER)，研究發現可預防紫外線(UV)輻射對於小鼠眼角膜的傷害與水晶體的病變，具體的成果已發表 [刊登於 2011 Molecular Vision期刊(SCI)][6,7]。

(b) 硫辛酸 (α-lipoic acid)，研究發現可預防紫外線(UV) 輻射對於小鼠眼球表面造成退化性傷害，同時可減緩乾眼與眼結膜杯狀細胞的退化的問題 [刊登於 2013 年IOVS期刊(SCI)][8]。

(c) 銅鑼杭菊(chrysanthemum morifolium flowers)，近期本實驗室以生物模式認證的方法，協助農糧署開發銅鑼杭菊用於護眼功效 (2012)，確認杭菊萃取物可用於有預防紫外線(UV)輻射所造成眼角模退化性傷害、乾眼與眼結膜杯狀細胞退化的功效，並找出生物有效食用劑量，協助以銅鑼杭菊為材料用於評估開發生技產品 [Molecular Eye protective effects of water soluble extract of chrysanthemum morifolium flowers] [9]。

(d) 銀杏葉萃取物(Ginkgo biloba extract)，本年度之一年期的國科研究計劃(101 年度)的實驗成果已確認具有護眼睛的功效，銀杏葉萃取物(Ginkgo biloba extract)具有減緩UVB誘導眼角膜組織退化的能力(NSC 101-2320-B-040-012-) [目前此研究成果專利報告已著手進行申請審核評估] [10] [19]。

(二) 結果與討論：

Ginkgo biloba extract (GK) protects against UVB-induced corneal damages

The effects of UVB exposure on the cornea surface were first examined. Damages on the corneal smoothness were seen in the UVB group, but not in the blank control group. As an indication for damages to the corneal smoothness, the light circle seen on the corneal surface became irregular after exposure to UVB (Fig. 2), in contrast to the regular circle seen in the control group (Fig. 2). With Ginkgo biloba extract (GK) intake, the irregularity was significantly ameliorated, particularly in the UVB/GK (2 mg/kg) group and UVB/GK (10 mg/kg) group (Fig. 2). Quantitative analysis showed that the mean scores of corneal smoothness were significantly reduced in the UVB/GK (2 mg/kg) group ($p<0.001$) and the UVB/GK (10 mg/kg) group ($p<0.001$) as compared with that in the UVB group (Fig. 2). Besides, Ginkgo biloba extract (GK) intake helped to reduce the opacity in the cornea after UVB exposure (Fig. 2). As shown in negative images, a broad area of opacity was routinely seen in the eye from the UVB group (Fig. 2), which was not found in the blank control group

(Fig. 2), and Ginkgo biloba extract (GK) helped to reduce cornea opacity after UVB exposure, particularly in the UVB/GK (2 mg/kg) group and UVB/GK (10 mg/kg) group. The reduction of corneal opacity was also demonstrated by quantitative analysis (Fig. 2). Another way to characterize the UVB-induced damages on the corneal surface was to detect the devitalized epithelial areas after lissamine green staining. With UVB exposure, a large patch of blue stain could be seen, as represented by Fig in Result 2. In contrast, no blue devitalized epithelial area was found in the eyes from the blank control group (Fig. 2). Evident reduction of blue stain area was found after Ginkgo biloba extract (GK) intake, particularly in their corresponding negative images. Quantitatively, significant reduction of lissamine green staining areas was also demonstrated in the UVB/GK (2 mg/kg) and the UVB/GK (10 mg/kg) groups, when compared with the UVB group (Fig. 2).

Reduction of UVB-induced polymorphonuclear leukocyte infiltration and MMP-9 expression by Ginkgo biloba extract (GK)

To further characterize the protective effects of Ginkgo biloba extract (GK) against UVB-induced corneal damages, histological analysis was performed. The results showed significant thinner corneal epithelial layer with UVB exposure (Fig.3-A) than without UVB exposure (Fig. 3-A). The epithelial cells generally exhibited some vacuoles after UVB exposure, indicating occurrence of cell death. Ginkgo biloba extract (GK) helped to prevent the reduction of corneal epithelial thickness. When compared with those in the UVB group and UVB/DMSO-PEG400 group (Fig. 3-A), the tissue sections from the eyes of the UVB/GK (0.4 mg/kg), UVB/GK (2 mg/kg) and UVB/GK (10 mg/kg) groups were found to have thicker epithelial layer in the central cornea. After UVB irradiation, the corneal stroma was extensively infiltrated by polymorphonuclear (PMN) leukocytes in the UVB group and UVB/DMSO-PEG400 group (Fig. 3-A). In contrast, significant reduction of PMN leukocyte infiltration was observed with dietary Ginkgo biloba extract (GK) intake at 2 mg/kg or at 10 mg/kg of body weight (Fig. 3-A). Quantitative analysis confirmed such reduction. The number of infiltrative PMN leukocytes was significantly reduced in the UVB/GK (0.4 mg/kg) group ($p<0.001$), UVB/GK (2 mg/kg) group ($p<0.001$) and the UVB/GK (10 mg/kg) group ($p<0.001$) as compared with that in the UVB group and UVB/DMSO-PEG400 group (Fig. 3-D).

Significant increase of MMP-9+ polymorphonuclear leukocytes (PMN) in the cornea of the UVB group and the UVB/DMSO-PEG400 group was detected as compared to that of the blank control group (Fig. 4-A). The accumulation of MMP-9+ polymorphonuclear leukocytes (PMN) in the corneal stroma as reflected by immunohistochemical analysis was attenuated in a dose-related manner with Ginkgo biloba extract (GK) intake (Fig. 4-A). Quantitative analysis confirmed such reduction. The number of infiltrative MMP-9+ PMN leukocytes was significantly reduced in the UVB/GK (2 mg/kg) group ($p<0.001$) and the UVB/GK (10 mg/kg) group ($p<0.001$) as compared with that in the UVB group and UVB/DMSO-PEG400 group (Fig. 4-B).

Ginkgo biloba extract (GK) helps to maintain P63+ corneal basal cells with inhibition of UVB-induced proinflammatory factors and Fas protein

UVB irradiation is the most critical factor in the pathogenesis of photokeratitis, since most of its energy is absorbed by the cornea [15-18].When eyes are excessively exposed to UVB, the oxidative stresses will be induced to a phototoxic level, leading to activation of inflammatory factors such as NF-κB and COX-2 Excessive UVB exposure also poses a risk to the conjunctival goblet cells. Therefore, shields to protect from UVB irradiation have to be used to minimize UVB exposure.

To further understand the protective effects of Ginkgo biloba extract (GK), we examined P63+ corneal basal cells to see whether the corneal repair capacity had been maintained. Decreased P63+ basal cell density was seen in the cornea exposed to UVB in the UVB group and the UVB/DMSO-PEG400 group (Fig. 5-A); in contrast to the normal distribution of P63+ basal cells in the blank control cornea. With Ginkgo biloba extract (GK) at 2 mg/kg and 10 mg/kg of body weight, the density of P63+ cells was close to that of the blank control group (Fig. 5-A). Quantitatively, the mean P63+ cell number was significantly increased in the UVB/GK (2 mg/kg) group and in the UVB/GK (10 mg/kg) group as compared with that in the UVB group and the UVB/DMSO-PEG400 group (Fig. 5-A). Besides, immunohistochemical staining was performed to examine the expression of two proinflammatory factors, NF-κB-p65 and COX-2, and the cell death regulator protein, the Fas protein to further explain the protective effects of Ginkgo biloba extract (GK). High level of NF-κB-p65, COX-2 and Fas (Fig. 5-B) expression was seen in the cornea exposed to UVB in the UVB group and the UVB/DMSO-PEG400 group, which was not observed in the blank group (Fig. 5-B). The expression of NF-κB-p65, COX-2 and Fas protein was attenuated in a dose-related manner with Ginkgo biloba extract (GK) intake (Fig. 5-B).

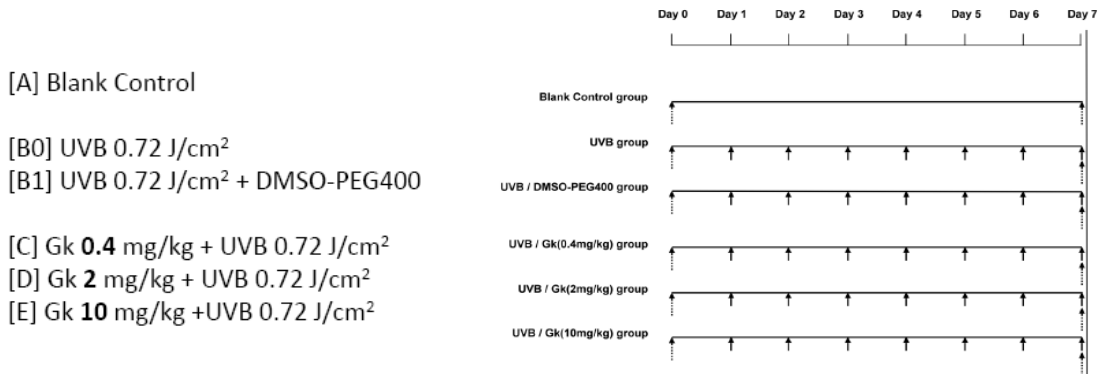
Ginkgo biloba extract (GK) maintains conjunctival goblet cell density under the influence of UVB Exposure

The conjunctival epithelium was examined before and after UVB irradiation, and its status with or without Ginkgo biloba extract (GK) treatment was compared. In the control group, the conjunctival surface consists of a cuboidal basal cell covered by two layers of epithelial cells flattened toward the free surface and not cornified. To confirm the protective effect of dietary α-LA on conjunctiva, the conjunctival goblet cell density was examined after PAS staining (Fig.6-A). The results showed that UVB irradiation caused degeneration of PAS-positive goblet cells. The number of goblet cells in the fornix conjunctiva was significantly lower in the UVB group and the UVB/DMSO-PEG400 group (Fig. 6-A) than that in the control group (Fig.6-A). When Ginkgo biloba extract (GK) treatment was given at 2 mg/kg or at 10 mg/kg of body weight after UVB exposure, the number of conjunctiva goblet cells was higher maintained (n = 5), respectively. Quantitative analysis showed that (1) the number of conjunctiva goblet cells was significantly maintained in the groups of UVB/GK (2 mg/kg) and UVB/GK (10 mg/kg); as compared with that of the UVB group and

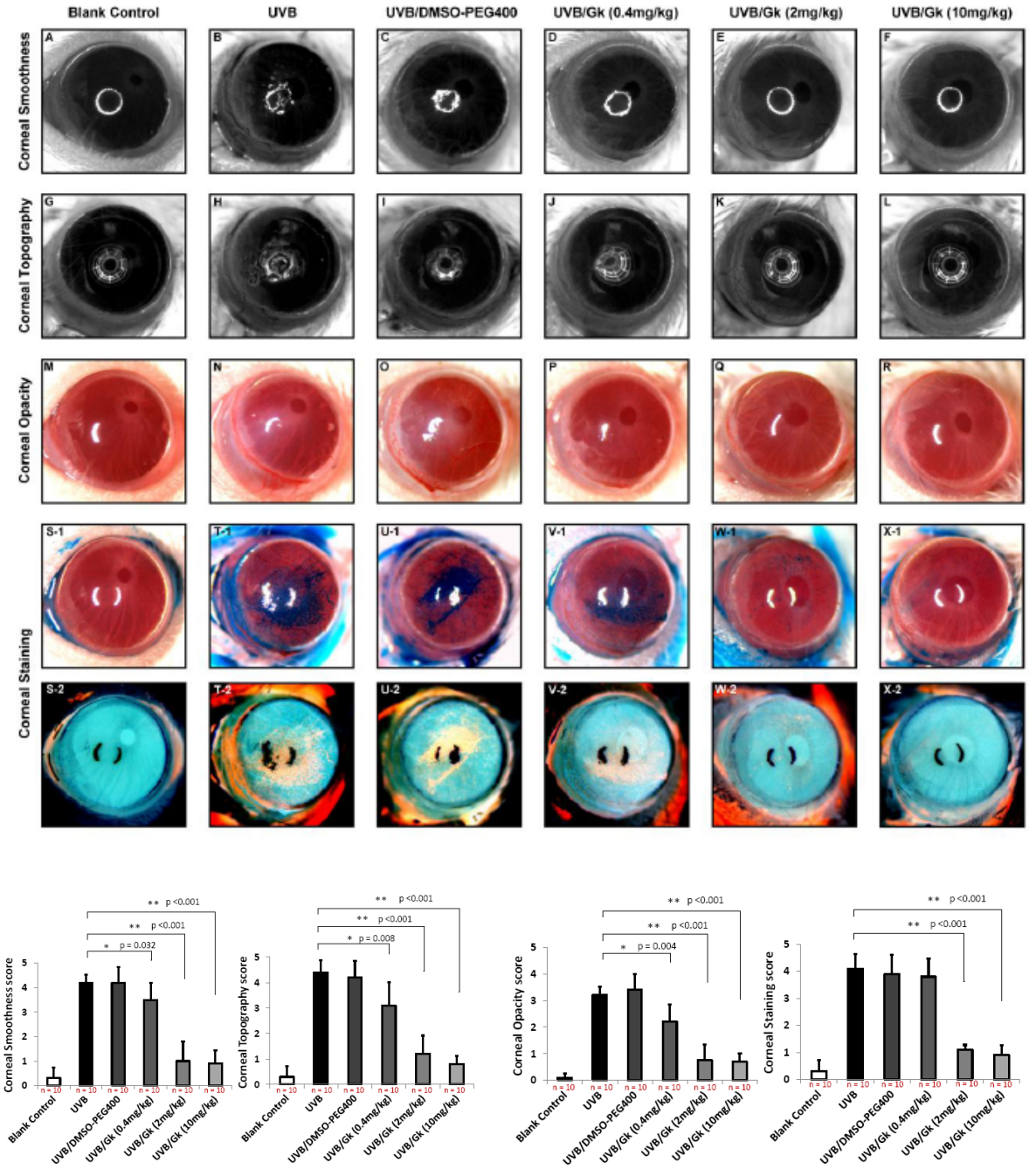
the UVB/DMSO-PEG400 group. (2) The size of conjunctiva goblet cells was also significantly maintained in the groups of UVB/GK (2 mg/kg) and UVB/GK (10 mg/kg). (Fig. 6-B)

We conclude that dietary Ginkgo biloba extract (GK), given sufficient dose, is effective to protect against UVB-induced photokeratitis and subsequent corneal and conjunctival degeneration, probably through multiple mechanisms other than anti-oxidative stresses. Given that Ginkgo biloba extract (GK) has been used for years as a dietary supplement for other purposes, the extra beneficial effects against UVB-induced ocular surface damages may be considered [10].

[Figure-1] Experimental groups and study design.

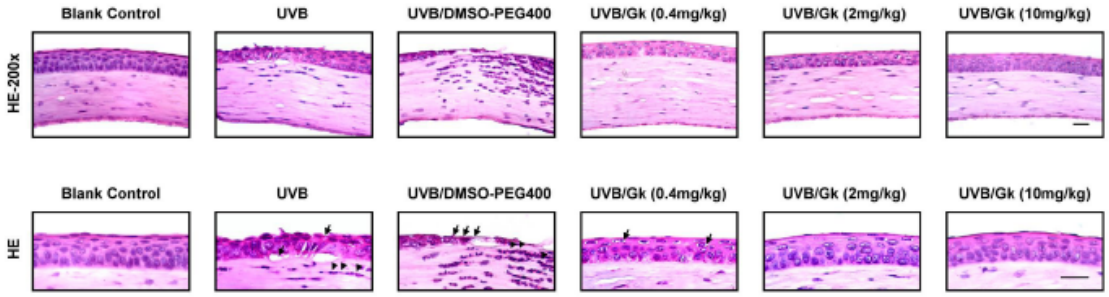


[Figure-2] Corneal surface evaluation among the experimental groups. The corneal smoothness (A-F), corneal topography (G-L), corneal opacity (M-R), and lissamine green staining (S-X) among the five study groups were assessed. Quantitative analysis of the corneal smoothness, corneal topography, opacity, and lissamine green staining among these study groups. After UVB irradiation in the UVB groups and the UVB/DMSO-PEG400 groups, showed disordered corneal smoothness and corneal topography, more corneal opacity degeneration, and more staining. The results showed that all the scores of corneal smoothness, corneal opacity, corneal opacity, and fluorescein staining were reduced by Ginkgo biloba extract (GK) in a dose-related manner. The results showed that the scores were more significant reduced in the groups with Ginkgo biloba extract (GK) at 2mg/kg and 10mg/kg. All p values were determined by the Kruskal Wallis test as compared to the UVB group.

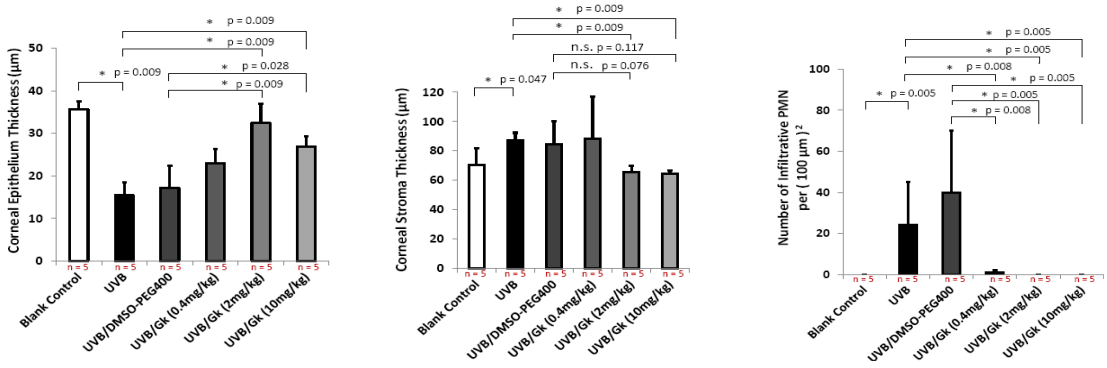


[Figure-3] UVB-induce reduction of central corneal epithelial thickness. (A) Hematoxylin-Eosin (HE) staining showed disordered stroma, thinner epithelium layer, more apoptotic vacuoles in the epithelium layer, more infiltration of polymorphonuclear leukocytes (PMN) after UVB irradiation in the UVB groups and the UVB/DMSO-PEG400 groups. These UVB-induced effects were reduced by Ginkgo biloba extract (GK) in a dose-related manner. (B) Quantitative analysis of central corneal epithelial thickness. (C) Quantitative analysis of corneal stroma thickness. (D) Quantitative analysis of infiltration of polymorphonuclear leukocytes (PMN). The p values were determined by the Wilcoxon-Mann-Whitney test as compared to each group.

(A)

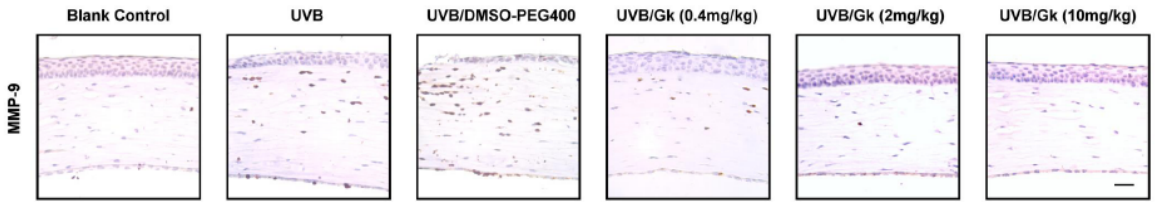


(B) **(C)** **(D)**

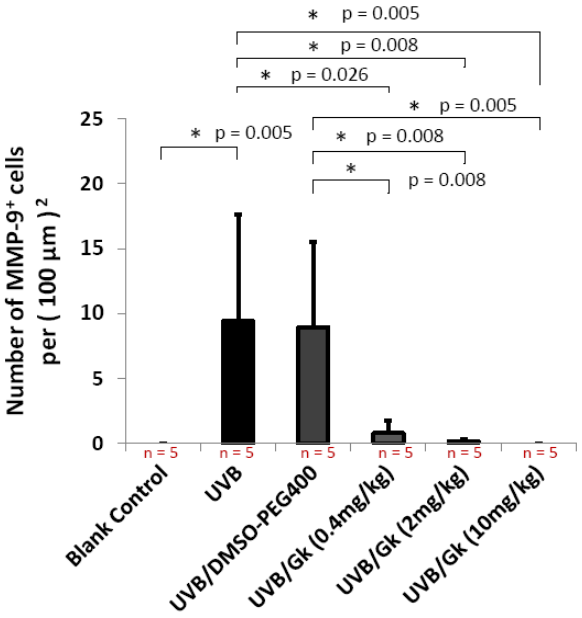


[Figure-4] Ginkgo biloba extract (GK) helps to inhibit UVB-induced infiltration of polymorphonuclear leukocytes in the cornea. Immunohistochemical analysis showed more infiltration of MMP-9+ polymorphonuclear leukocytes (PMN) in the corneal stroma after UVB irradiation in the UVB groups and the UVB/DMSO-PEG400 groups. (A) These UVB-induced effects were reduced by Ginkgo biloba extract (GK) in a dose-related manner. Staining analyses revealed the protective effects in corneal thickness and reduction of MMP-9+ polymorphonuclear leukocytes (PMN) in the groups with Ginkgo biloba extract (GK) at 0.4mg/kg, 2mg/kg and 10mg/kg. (B) Quantitative analysis of infiltration of MMP-9+ polymorphonuclear leukocytes (PMN). The p values were determined by the Wilcoxon-Mann-Whitney test as compared to each group.

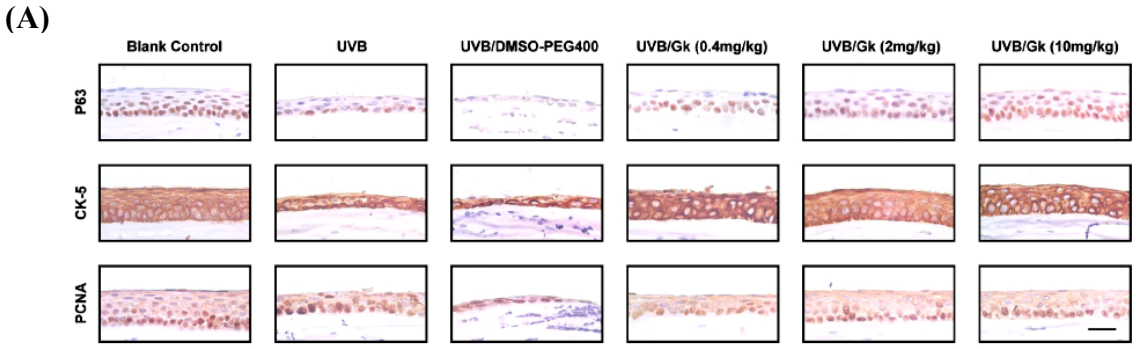
(A)

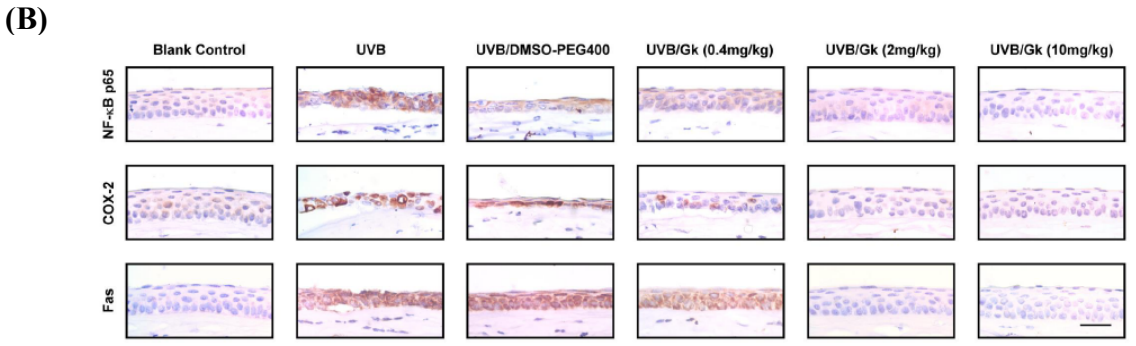


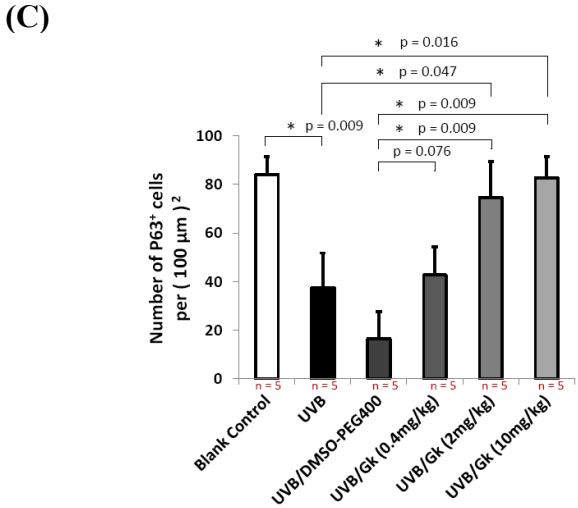
(B)



[Figure-5] Ginkgo biloba extract (GK) helps to maintain P63+ corneal basal cells, maintain CK5+ corneal cells, maintain PCNA+ corneal cells and inhibit UVB-induced proinflammatory factor and cell death protein expression. In (A), immunohistochemical analysis showed severe loss of P63+ basal cells, loss of CK-5+ corneal cells, and loss of PCNA+ corneal cells after UVB irradiation in the UVB groups and the UVB/DMSO-PEG400 groups. These UVB-induced effects were reduced by Ginkgo biloba extract (GK) in a dose-related manner. In (B), immunohistochemical analysis showed increased expression of NF−κB, COX-2 and Fas protein after UVB irradiation in the UVB groups and the UVB/DMSO-PEG400 groups. These UVB-induced effects were reduced by Ginkgo biloba extract (GK) in a dose-related manner. In (C), quantitative analysis of P63+ basal cells in the corneal epithelium among the study groups. Ginkgo biloba extract (GK) helped to maintain P63+ basal cells after UVB exposure in a dose-related manner. The p values were determined by the Wilcoxon-Mann-Whitney test as compared to each group.

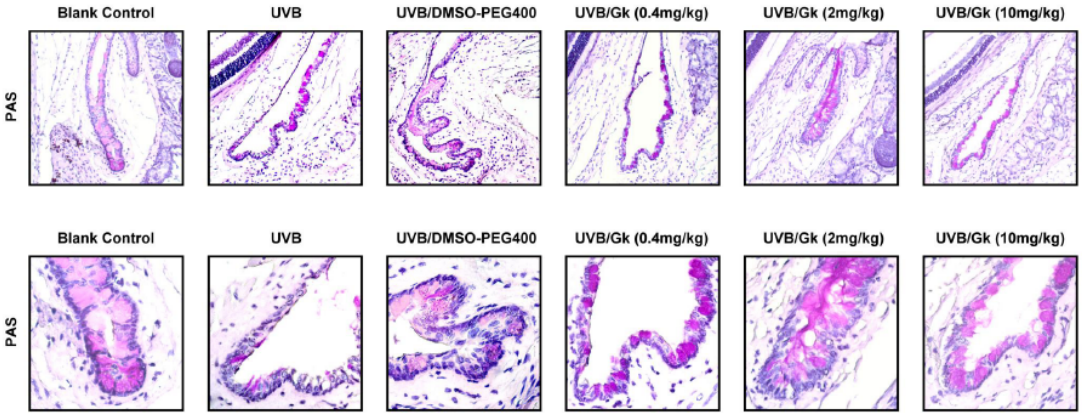




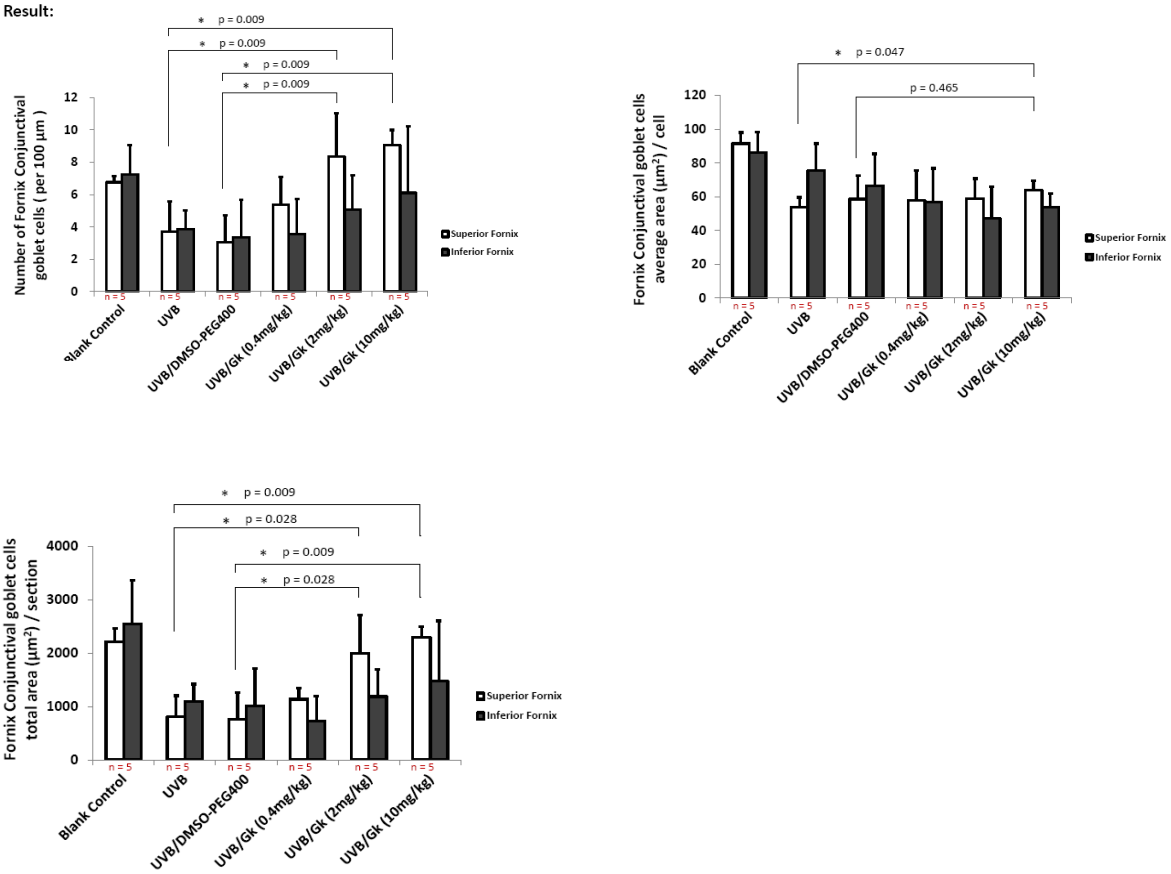


[Figure-6] Ginkgo biloba extract (GK) maintains conjunctival goblet cell density under the influence of UVB exposure In (A), typical squamous metaplasia was found in the fornix conjunctiva after UVB irradiation, which was prevented by Ginkgo biloba extract (GK). Conjunctival goblet cells as indicated by the pink PAS positive stain were significantly reduced in the UVB group, but were maintained by Ginkgo biloba extract (GK) in a dose-related manner even after UVB irradiation. In (B), quantitative analysis of goblet cell density among the study groups (n=5). The p values were determined by the Wilcoxon-Mann-Whitney test as compared to the UVB group.

(A)



(B)



(三) 計畫討論與建議：

◎ 銀杏葉萃取物：銀杏葉目前在國際醫藥市場上，已是重要的商品原料，有相當的市場價值。橫跨「保健」與「醫療」市場的兩端。在醫療功能方面顯示，銀杏葉製劑目前對於老年失智症的防治效果較被肯定，可以有效增加腦血流量。銀杏葉萃取物可改善正常人或動物的記憶和認知能力，對不同程度智障的阿爾茨海默病（AD）患者的認知能力和社會生活能力均有一定改善作用[11, 12]。除了抗氧化防護效果外，銀杏葉製劑對於其他退化性疾病的防護仍有待進一步論證 [13]。視網膜神經組織相似於大腦，因此推論銀杏葉製劑應有機會可用於預防或改善視網膜的問題，但實際的情形有待後續本化的實驗來認證。銀杏葉萃取物之主要生物活性成份為：銀杏黃酮配糖體 (Flavone glycosides)、銀杏內酯 (Ginkgolides)、山奈酚(Kacmpferol)、與槲皮素(Quercetin)等。銀杏葉所發揮的功效被認為是整性，即其中的多種成分聯合引起的療效比任何單獨的壹個成分強得多[14]。

◎ 於傳統醫學的處方，銀杏葉萃取物(Ginkgo Biloba Extract)已經被拿來用於臨床上預防視覺的退化，在NCBI PubMed 可找到一些文獻紀錄，至今有50篇。銀杏葉萃取物(Ginkgo Biloba Extract)被認為用於減緩青光眼(Glaucoma)病患的視覺退化、維持眼球血管的通透性、增加預後視覺的品質有實質幫助，但亦有學者持相反的意見。可能基於銀杏葉萃取物(Ginkgo Biloba Extract)對於護眼保健的相關機轉探討及其護眼功能性生理分析，目前資料非常缺乏；因此，在這「護眼保健」方面的研究，未來有相當大的發展空間。另外，銀杏葉萃取物(Ginkgo Biloba Extract)是否亦可應用於預防紫外線、LED光..等有害光輻射所造成的眼病 ？或應用於「減緩」或「反轉」因科技文明所帶來的退化性視覺疾病呢 ？雖然目前在NCBI PubMed 尚無文獻紀錄，但是從本計畫實驗初步的結果，我們認為後續的研究有相當高的可行性，並認為銀杏葉萃取物(Ginkgo Biloba Extract)所能帶來的預期 ”保健效果” 與 “經濟發展效益” 是相當的可觀。

◎ 近年來台灣的教育推廣多媒體教材的使用，但是3C電子產品的過度使用後，對於視覺組織的威脅與防範是值得省思的。亞太地區工商業發達，根據統計國人一天工作時數長達8~12小時，同時長時間處於視覺終端處理的行為(Visual Display Terminal)，對視覺健康影響甚鉅。在快速LED、數位螢幕科技的進步背後，是否有察覺為什麼罹患白內障、青光眼、眼中風、視網膜病變、老年性黃斑部病變的年齡層以快速的腳步往前提呢 ？事實上，近代醫學科學研究已提出警訊，證實動物在強光或劣質LED光源下生活容易造成視網膜的病理損傷與視覺的退化。近代臨床醫學亦提出嚴重的警訊，眼睛疾病的罹患率有年輕化的趨勢。因此，相對的可預期護眼保健的市場於未來會有相當大的成長空間，開發「護眼保健素材」應是未來生技發展的新趨勢。現代社會，人群生活中眼睛長時間固定在某一焦距，已經見怪不怪，會出現的症狀：眼睛痠、眼睛紅、流淚、乾澀、脹痛，甚至眼窩痛，有噁心嘔吐感或合併視力衰退等症狀。這些可能會發生的視覺問題事情，是否可以藉由來視覺保健食品避免呢? 或可「反轉」退化後的視覺品質 ？銀杏葉萃取物及其活性成份，可能為一種好的選擇，若能用於護眼保健，會具有相當的市場規模。可藉由未來研究計畫研究找出銀杏葉萃取物及其活性成份的護眼功效，預期直接用於醫療生技廠業的發展會有相當大的幫助。

◎ 綜觀台灣目前「健康食品」的認證和控管，在護肝、調節血脂、不一形成體脂肪..等方面的動物實驗認證規範較為完善，唯獨缺乏「護眼保健」這一系列，可能源自於過去動物實驗模式所提供的支援較為限制。然而，現今護眼相關保健食品琳瑯滿目，不恰當的商業廣告充斥於市面，事實上不見得會有確切的效果。因此，目前對於「護眼保健」這一系列的健康食品之管理所面臨的挑戰已經迫在眉睫。我們認為本研究計畫之動物實驗評估技術層面具有相當的發展價值，規劃於探討此計畫的研究目的之同時，額外評估此動物模式用以認證「護眼健康食品」的效益，有助於未來政府持續推動「健康食品的認證」。

(四) 研究方法：

(1) 實驗動物方法：

Study groups, ultraviolet B irradiation, and Ginkgo biloba extract (GK) treatment: The mice were randomly split into 6 groups, including (1) Blank control (no UVB exposure, no α-LA treatment), (2) UVB (exposure to UVB, no GK treatment), (3) UVB/ UVB/DMSO-PEG400 (exposure to UVB, no GK treatment, with DMSO-PEG400 treatment),(4) UVB/ GK (0.4mg/kg) (exposure to UVB with GK treatment at 0.4 mg/kg body weight), (5) UVB/ GK (2mg/kg) (exposure to UVB with GK treatment at 2 mg/kg body weight), and (6) UVB/ GK (10 mg/kg) (exposure to UVB with GK treatment at 10 mg/kg body weight). To expose the corneas to UVB irradiation, the mice were anaesthetized with, with both of their eyes exposed to daily UVB light (CN-6, Vilber Lourmat, Germany) in a darkroom. Each daily UVB exposure was performed to reach a total amount of 0.72 J/cm2 within 10 min, with the entire UVB exposure course completed in a consecutive 7-day period. The peak wavelength of UVB light was 312 nm. For the groups with GK treatments, starting from day 0 (one day before UVB exposure) and terminated on day 7. The mouse body weight was readily maintained between 25 g – 27 g during the 8 day-period of experiment. All mice were sacrificed on day 8 for analysis. All experiment protocols were reviewed and approved by the Animal Care and Use Committee of Chung Shan Medical University and were performed in agreement with the Association for Research in Vision and Ophthalmology (ARVO) Resolution on the Use of Animals in Research [6-10].

(2) 實驗動物評估方法：

實驗結束後，將小鼠麻醉，以我們視光實驗室的設備執行角膜的平滑度(Corneal smoothness text)、角膜的透光度(Corneal opacity text)、角膜染色的程度(Corneal staining text) 和水晶體的透光度(Lens opacity text)的檢驗[6-10]。分級評分系統如下：

(a) Corneal smoothness scores：Based on the digital images, the corneal smoothness scores were determined by using a 5-point scale based on the number of distorted quadrants in the reflected ring: 0, no distortion; 1, distortion in 1 quadrant of the ring (3 clock hours); 2, distortion in 2 quadrants (6 clock hours); 3, distortion in 3 quadrants (9 clock hours); 4, distortion in all 4 quadrants (12 clock hours); and 5, severe distortion, in which no ring could be recognized.

(b) Corneal opacity scores：images were taken and graded from 0 (normal) to 4 (severe) in all corneas through a stereoscopic zoom microscope (SMZ 1500; Nikon). An opacity lesion score system was used: 0, normal cornea; 0.5, mild haze only under dissection microscope; 1, mild haze; 2, moderate haze with visible iris; 3, severe haze with invisible iris; 4, severe haze with corneal ulceration.

(c) Staining scores were determined according to a grading system based on area of stain in the cornea. The total area of punctuate staining was designated as grade 0; grade 1, less than 25% of cornea stained with scattered punctuate staining; grade 2, 25%–50% of cornea stained with diffuse punctuate staining; grade 3, 50%–75% of cornea stained with punctuate staining and apparent epithelial defects; grade 4, more than 75% of cornea stained with abundant punctuate staining and large epithelial defects.

(3) Statistical analysis:

All data were obtained from triple repeats and are presented as the means ± standard error of the means (SEMs) and were compared among groups. Corneal smoothness scores、Corneal opacity scores、Staining scores、the retinal thickness and the means of labeled retinal cells were compared and analyzed by Mann Whitney test. All statistical analyses were performed by using the Prism program (GraphPad Software, San Diego, CA, USA). [6-10]

(4) Histopathological analysis and immunohistochemistry:

Following assessment of corneal damages, the mice were sacrificed by cervical dislocation. One of the two eyes from each mouse was randomly selected and extracted. The extracted eyes, including the eye lids, were processed for Hematoxylin-Eosin (HE) stain following conventional procedures.10, 31-33 For immunohistochemistry, the tissue sections were boiled in citrate buffer (pH 6.0) for 20 min for antigen retrieval and then incubated respectively with one of the following antibodies: mouse anti-P63 (1/50, cat. no. sc-8431; Santa Cruz Biotechnology, Santa Cruz, CA. USA), or rabbit anti-NF-kB-p65 (1/200, cat. no. E379; Epitomics, PA. USA), or rabbit anti-COX-2 (1/100, cat. no. ab15191; Abcam, PA. USA), or rabbit anti-Fas antibody (1/100, Abcam, PA. USA) or rabbit anti-MMP9 (1/100, cat. no. ab38898; Abcam, Cambridge, MA. USA) The preparations were then incubated with a horseradish peroxidase-conjugated secondary antibody (1/200), either anti-mouse or anti-rabbit IgG (Jackson ImmunoResearch Laboratories, Inc., PA. USA), followed by washes and incubation in diaminobenzidine tetrahydrochloride solution for color detection, and counterstain with hematoxylin [8].

(5) Goblet Cell Density assay:

Periodic acid-Schiff (PAS) stain was performed to evaluate conjunctival epithelial morphology and the number of goblet cells in the inferior conjunctiva was counted. The counting of goblet cells was performed under a microscope (ECLIPSE E100; Nikon, Melville, NY), with a 20× objective. Five different sections were randomly selected for counting and an average was calculated. All scorings were performed by 2 observers without

prior knowledge of the UVB exposure and study groups [8].

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(六) 計畫成果：

研討會論文

(1) The Chemopreventive Effects of Ginkgo biloba Extract Against UVB-induced Corneal Phototoxicity. 陳伯易#., et al (2013) (2013 第 28 屆生物醫學聯合學術年會)

國科會補助計畫衍生研發成果推廣資料表

日期:2013/10/29

國科會補助計畫	計畫名稱: 探討銀杏葉萃取物及其相關活性成份對傷害性光線誘發小鼠眼組織損傷退化之保護效果
	計畫主持人: 陳伯易
	計畫編號: 101-2320-B-040-012- 學門領域: 保健營養

無研發成果推廣資料

101 年度專題研究計畫研究成果彙整表

計畫主持人：陳伯易	計畫編號：101-2320-B-040-012-
計畫名稱：探討銀杏葉萃取物及其相關活性成份對傷害性光線誘發小鼠眼組織損傷退化之保護效果	

成果項目			量化			單位	備註（質化說明：如數個計畫共同成果、成果列為該期刊之封面故事...等）
			實際已達成數（被接受或已發表）	預期總達成數(含實際已達成數)	本計畫實際貢獻百分比		
國內	論文著作	期刊論文	0	0	100%	篇	
		研究報告/技術報告	1	1	100%		
		研討會論文	1	1	100%		
		專書	0	0	100%		
	專利	申請中件數	1	1	100%	件	
		已獲得件數	0	0	100%		
	技術移轉	件數	0	0	100%	件	
		權利金	0	0	100%	千元	
	參與計畫人力（本國籍）	碩士生	5	5	100%	人次	
		博士生	0	0	100%		
		博士後研究員	0	0	100%		
		專任助理	0	0	100%		
國外	論文著作	期刊論文	1	1	100%	篇	
		研究報告/技術報告	0	0	100%		
		研討會論文	0	0	100%		
		專書	0	0	100%	章/本	
	專利	申請中件數	0	0	100%	件	
		已獲得件數	0	0	100%		
	技術移轉	件數	0	0	100%	件	
		權利金	0	0	100%	千元	
	參與計畫人力（外國籍）	碩士生	0	0	100%	人次	
		博士生	0	0	100%		
		博士後研究員	0	0	100%		
		專任助理	0	0	100%		

其他成果 (無法以量化表達之成果如辦理學術活動、獲得獎項、重要國際合作、研究成果國際影響力及其他協助產業技術發展之具體效益事項等，請以文字敘述填列。)	綜觀台灣目前「健康食品」的認證和控管，在護肝、調節血脂..等方面的動物實驗認證規範較為完善，唯獨缺乏「護眼保健」這一系列，可能源自於過去動物實驗模式所提供的支援較為限制。然而，現今護眼相關保健食品琳瑯滿目，不恰當的商業廣告充斥於市面，事實上不見得會有確切的效果；目前對於「護眼保健」這一系列的健康食品之管理所面臨的挑戰已經迫在眉睫。於後 3C 面板電子產品的時代，視覺退化的風險遠遠高於近視與老花的問題。因此，我們認為本研究「動物實驗評估技術模式」具有相當的發展價值，探討此計畫研究目的同時，額外評估此動物模式用以認證「護眼健康食品」的效益，有助於未來政府持續推動「健康食品的認證」。本計劃提供了與視覺保健相關的實驗動物模式，可做為將來政府推動「護眼健康食品認證」法案時的重要參考依據。

	成果項目	量化	名稱或內容性質簡述
科教處計畫加填項目	測驗工具(含質性與量性)	0	
	課程/模組	0	
	電腦及網路系統或工具	0	
	教材	0	
	舉辦之活動/競賽	0	
	研討會/工作坊	0	
	電子報、網站	0	
	計畫成果推廣之參與（閱聽）人數	0	

國科會補助專題研究計畫成果報告自評表

請就研究內容與原計畫相符程度、達成預期目標情況、研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性）、是否適合在學術期刊發表或申請專利、主要發現或其他有關價值等，作一綜合評估。

1. 請就研究內容與原計畫相符程度、達成預期目標情況作一綜合評估

■達成目標

□未達成目標（請說明，以 100 字為限）

□實驗失敗

□因故實驗中斷

□其他原因

說明：

2. 研究成果在學術期刊發表或申請專利等情形：

論文：□已發表 ■未發表之文稿 □撰寫中 □無

專利：□已獲得 ■申請中 □無

技轉：□已技轉 ■洽談中 □無

其他：(以 100 字為限)

3. 請依學術成就、技術創新、社會影響等方面，評估研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性）(以 500 字為限)

近年來國人視覺健康逐年下降，同時視覺退化疾病有逐年增加的趨勢，其中又以角結膜的退化最為常見。其問題可歸咎於現代環境中的傷害性輻射光源，如: 紫外線。但是長期臨床上的觀察，可發現以光學鏡片阻隔的效果不盡完全。視覺退化問題一旦發現時，多半視力已嚴重的降低，即使接受了最先進治療，視覺品質重建與視力恢復並不理想，如: 紫外線造成角膜不平整退化與散光後，視覺品質會嚴重降低，不易回復。本計劃探討銀杏葉萃取物用於預防紫外線退化性眼角膜疾病的防治，並討論機轉及其功能性成份的保健效果。研究成果發現，銀杏葉萃取物於小鼠可能預防 UVB 誘導眼角膜與結膜的退化，如: 預防角膜表面的不平整退化，預防角膜表面不透明變性與角膜上皮細胞損傷退化，預防結膜杯狀細胞的委縮。實驗結果發現，銀杏葉萃取物可藉由抑制 NF-κB、COX-2、MMP-9 蛋白質的表現來預防 UVB 誘導眼球表面的退化。

本計劃因經費補助的額度與期限的限制，集中於紫外線防護部份的探討。本研究成果，對於退化性眼球表面疾病的防治、機轉及其銀杏葉萃取物功能性成份的保健效果提供參考資訊，以供生技產業的發展。後續建議開發小鼠功能性視覺評估模式，更深一層的探討銀杏葉萃取物於「護眼保健」的功效，如：用於預防藍光傷害的功能性視覺評估。此亦為我們研究室持續發展的方向。

綜觀台灣目前「健康食品」的認證和控管，在護肝、調節血脂..等方面的動物實驗認證規範較為完善，唯獨缺乏「護眼保健」這一系列，可能源自於過去動物實驗模式所提供的

支援較為限制。然而，現今護眼相關保健食品琳瑯滿目，不恰當的商業廣告充斥於市面，事實上不見得會有確切的效果；因此，目前對於「護眼保健」這一系列的健康食品之管理所面臨的挑戰已經迫在眉睫。於後 3C 面板電子產品的時代，視覺退化的風險遠遠高於近視與老花的問題。因此，我們認為本研究「動物實驗評估技術模式」具有相當的發展價值，探討此計畫研究目的同時，額外評估此動物模式用以認證「護眼健康食品」的效益，有助於未來政府持續推動「健康食品的認證」。本計劃提供了與視覺保健相關的實驗動物模式，可做為將來政府推動「護眼健康食品認證」法案時的重要參考依據。