

Effects of *Potentilla fulgens* on the Changes Made in the Retinal Damage Induced by Traumatic Head Injury

Efectos de *Potentilla Fulgens* en los Cambios Producidos Debido al daño Retinal Inducido por Lesión Traumática en la Cabeza

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SUMMARY: Traumatic head injury is a leading cause of mortality and morbidity. As a result of head trauma occurring in the retina of the various biochemical, histological and immunohistochemical effects were investigated. Sprague–Dawley rats were subjected to traumatic brain injury with a weight-drop device using 300 g-1 m weight–height impact. Twenty one rats were divided into three groups, as group 1 (vehicle-treated control), group 2 (vehicle-treated trauma) group 3 trauma + *Potentilla fulgens* (*P. Fulgens*) 400 mg/kg/day, i.p.). Distilled water was used as vehicle. All rats were decapitated 5 days after the induction of trauma, and the protective effects of *P. Fulgens* were evaluated by histological, immunohistochemical and biochemical analyses. Although further studies are necessary to evaluate the time-and dose-dependent neuroprotective effects of *P. Fulgens*. Depending on whether trauma inhibits apoptosis of photoreceptor cells, ganglion cells, it is thought that the the support against the degeneration of neural connections can be considered. This study indicates that P.Fulgens is potentially useful for the treatment of eye disorders induced by traumatic brain injury.

KEY WORDS: Traumatic injury; Retina; *Potentilla fulgens*; Rat.

INTRODUCTION

Traumatic brain injury (TBI) leads to a substantial number of deaths and cases of permanent disability. Many studies related to traumatic brain injury have been performed. This traumatic damage is caused by damage to the retinal ganglion cell, outer nuclear layer, and neuroglia, and the effects on the eye and visual system are diminished (Mohan *et al.*, 2013; Zou *et al.*, 2013; Wang *et al.*, 2014). The authors found a decrease in retinal nerve fiber layer (RNFL) thickness and a decrease in pattern electroretinography (ERG) 3 to 4 months after injury, which correlated with punctate regions of reduced cellularity in the ganglionic layer and damage to the optic nerve (Mohan *et al.*) *Potentilla fulgens* is an alpine plant of Western Himalayas which is consumed in all parts of the world for its promising medicinal properties. Pharmacologically, the aerial and root portions of the plant are reported to have antioxidant (in vitro models), antitumor, hypoglycemic and anti-hyperglycemic activities (Syiem *et al.*, 2003; Syiem *et al.*, 2009; Jaitak *et al.*, 2010). The general signs and symptoms of toxicity, food and water intake and mortality rates of animals were observed within 72 h post-treatment. From these observations, lethal dose 50 % (LD50) was calculated using

SPSS software (Chen *et al.*, 2009). GFAP is a 50-52 kDa acidic cytoskeletal protein. It is a marker protein of colloid cell, with plenty and exclusive expression in it. GFAP can be used to mark the post trauma changes of colloid cell, and normally GFAP is under the dynamic adjustment of glia cells (Zhang *et al.*, 2004). Post trauma proliferated GFAP-positive colloid cell can promote caryocinesis of colloid cell, causing the differentiation of primitive progenitor cell towards mature glia cell. Various kinds of central nervous system lesions can induce colloid cell response (Austin *et al.*, 1995). Cadherins are a family of transmembrane glycoproteins that mediate calcium-dependent cell-to-cell adhesion in solid tissues. Numerous isoforms of cadherin have been identified in a tissue-specific manner and shown to regulate morphogenesis through homophilic binding (Nose *et al.*, 1988; Takeichi *et al.*, 1990). E-cadherin is responsible for stable cell-to-cell adhesion. The alteration in the E-cadherin molecule in the retinal cellular junction can play a role in visual function by affecting the cell-cell complex. *Potentilla fulgens* effects on changes in the retina after head trauma in the study were investigated by biochemical, histological and immunohistochemical methods.

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MATERIAL AND METHOD

The investigation was conducted in accordance with the Guide for the Care and Use of Laboratory Animals published by US National Institutes of Health (NIH Publication no. 85-23, revised 1996). All experimental protocols were approved by the Dicle University Animal Care and Use Committee. Male Sprague-Dawley rats (300–350 g) were housed in an air-conditioned room with 12-h light and dark cycles, where the temperature ($23\pm 2^\circ\text{C}$) and relative humidity (65–70 %) were kept constant. The animals were anesthetized by an intraperitoneal injection of 5 mg/kg xylazine HCl (Rompun, Bayer HealthCare AG, Germany) and 40 mg/kg ketamine HCl (Ketalar, Pfizer Inc., USA), and were allowed to breathe spontaneously. A rectal probe was inserted, and the animals were positioned on a heating pad that maintained the body temperature at 37°C . Briefly, a trauma device which works by dropping a constant weight from a specific height through a tube was used. Thirty minutes after the trauma, rats were injected either saline or *Potentilla fulgens* (400 mg/kg/day) via intraperitoneally. On the fifth day all the 40 animals were killed after TBI. Each group consisted of 10 animals. These rats were used for biochemical and histopathological

analysis. The MDA level and SOD, GSH-Px, and CAT activity were determined in each retina and the average values of each group were calculated

Statistical analysis. Statistical analysis was carried out using GraphPad Prism 4.0 software (GraphPad Software, 2003, San Diego, CA, USA). All data are presented as mean \pm standard deviation (SD). Groups of data were compared with an analysis of variance (ANOVA) followed by Tukey's multiple comparison tests. Values of $p < 0.05$ were considered as significant.

RESULTS AND DISCUSSION

When the trauma group was compared with the control and *P. Fulgens* groups, the MDA values were significantly increased ($p < 0.05$). Treatment with *P. Fulgens* significantly decreased the MDA levels compared with the trauma group ($p < 0.05$). There were no statistically significant differences between the control and *P. Fulgens* groups ($p = 0.488$). Following TBI, tissue GSH-Px levels decreased significantly when both the control and *P. Fulgens*

Table I. Biochemical results relevant to the study groups.

Groups	Control group	<i>Potentilla. Fulgens</i> group	Trauma	Trauma+ <i>Pot</i>
MDA (nmo I/g)	20.71 \pm 1.19	20.31 \pm 0.59	34.88 \pm 0.99*	27.71
GSH-Px (U/g)	24.17 \pm 0.43	24.44 \pm 0.58	15.69 \pm 0.63*	21.21
SOD (U/g)	0.89 \pm 0.028	0.90 \pm 0.022	0.58 \pm 0.018*	0.78 \pm
CAT (K/g)	1.60 \pm 0.003	1.59 \pm 0.006*	1.26 \pm 0.049*	1.45 \pm

Values are represented as mean \pm SD. MDA: malondialdehyde, GSH-Px: glutathione peroxidase, SOD: superoxide dismutase, CAT: Catalase * $p < 0.05$, versus control, + $p < 0.05$, trauma + *P. Fulgens* versus trauma

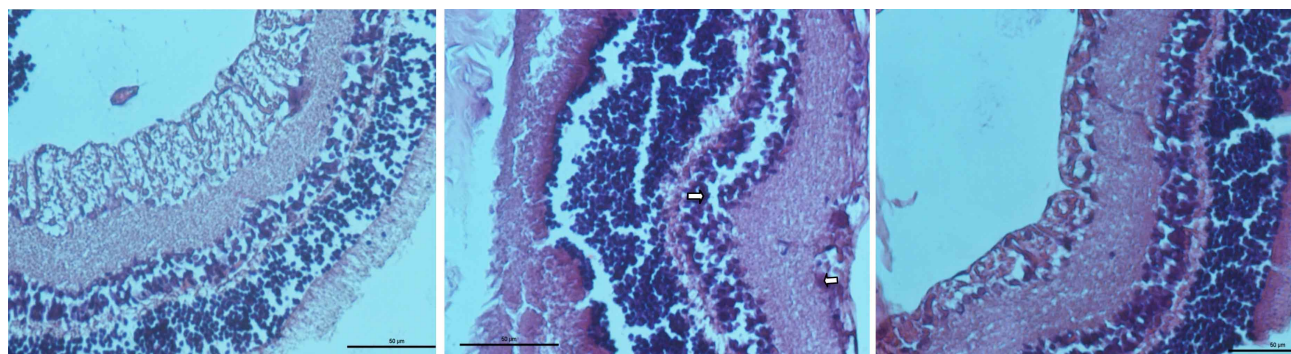


Fig. 1A .Control group: Normal appearance of the internal and outer nuclear layers and the normal organization of the retinal ganglionic layer. H-E staining Bar 50 μm , B-Trauma group, Organization and degeneration of cells in the inner and outer nuclear layers (arrow), dilation and hemorrhage in the blood vessels of the ganglionic layer (arrow), H-E staining Bar 50 μm . C. Trauma+*P.fulgens* group: Degeneration of cells in the inner and outer nuclear layers, degeneration of the inner and outer plexiform layers, fibrous structures regular shedding of the ganglionic layer, no dilatation and no bleeding in the blood vessels of the ganglionic layer, H-E staining Bar 50 μm ,

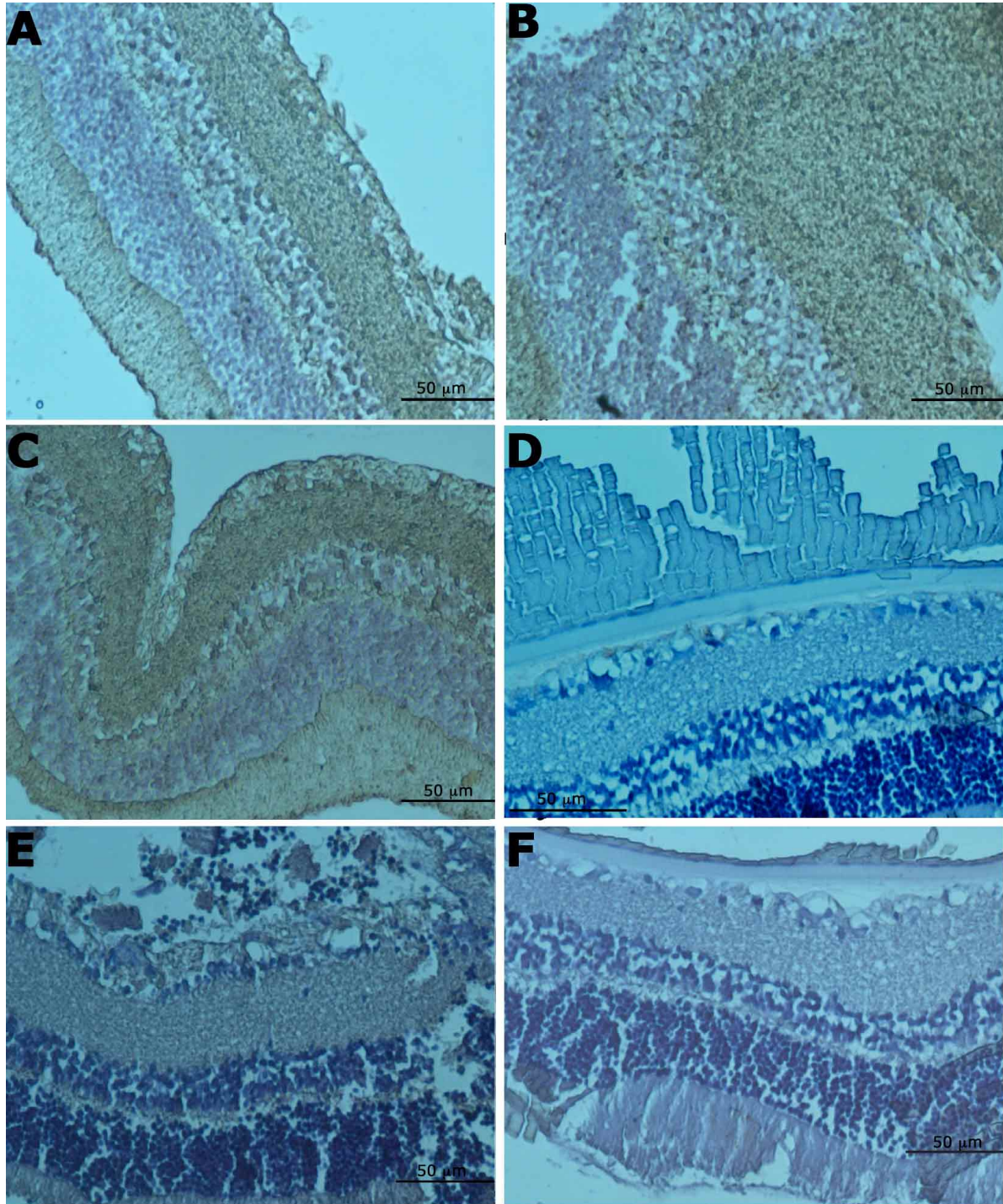


Fig. 2A. Control group: Regular distribution of E-cadherin expression in the ganglionic layer, regular internal and external plexiform layers in cells in the inner and outer nuclear layers, E-cadherin immunohistochemical staining Bar 50 µm. B. Trauma group: The separation in the cells in the inner and outer nuclear layers and the expression of E-cadherin is weak (arrow), E-cadherin immunohistochemical staining Bar 50 µm. C. Trauma+*P.fulgens* group: E-cadherin expression is positive in cells in the inner and outer nuclear layer, membranes and cellular connections, E-cadherin immunohistochemical staining Bar 50 µm, D. Control group: GFAP protein expression in the ganglionic layer and inner and outer layers plexiform GFAP immunohistochemical staining Bar 50 µm, E. Trauma group An increase in GFAP expression in the ganglionic layer,GFAP immunohistochemical staining Bar 50 µm. F. Trauma+*P.fulgens* group:Weak GFAP expression in plexiform layer and ganglion cells, GFAP immunohistochemical staining Bar 50 µm.

groups were compared with the trauma group ($p < 0.05$). Treatments with *P. Fulgens* significantly increased the tissue GSH-Px levels compared with the trauma group ($p < 0.05$). There were no also significant differences between the control and *P. Fulgens* groups ($p=0.383$). When the tissue SOD activities of the control and *P. Fulgens* groups were compared with that of the trauma group, a statistically significant difference was observed ($p < 0.05$); these data showed that after TBI, tissue SOD activity was decreased. Treatment with *P. Fulgens* significantly increased the tissue SOD activity ($p < 0.05$). There were no significant differences between the control and *P. Fulgens* groups ($p = 0.496$). Following TBI, tissue CAT activity decreased significantly when both the control and *P. Fulgens* groups were compared with the trauma group ($p < 0.05$). Treatments with *P. Fulgens* significantly increased the tissue CAT activity compared with the trauma group ($p < 0.05$). There were significant differences between the control and *P. Fulgens* groups ($p < 0.05$). The biochemical results of the study are summarized in Table I.

Traumatic brain injury (TBI) is significantly associated with the over-production of reactive oxygen species. Traumatic brain injury self-reported visual complaints and symptoms such as blurry vision and photosensitivity and visual impairment such as visual acuity loss and visual field defects are reported (Brahm *et al.*, 2009). Cadherins play distinctive roles in retinal regeneration. The differentiation between the signal paths of cells and has a fundamental role in the identification of cell-cell contact (Austin *et al.*; Dorsky *et al.*, 1997). Tzekov *et al.* (2014) observed that cellularity decreased in the inner retina layer and ganglionic layer after trauma. In our study, it was observed that the inner and outer nuclear layer cellular integrity decreased after trauma, and the integrity of membranes deteriorated (Fig. 2b). GFAP is the major protein of glial intermediate filaments in astrocytes. It has been proposed as a specific marker for assessing astrocytic response to injury. An increase in GFAP expression is a cardinal feature of many pathological conditions of the CNS and astrocytes (Hausmann *et al.*, 2000; Yu *et al.*, 2004). GFAP is a 51-kDa intermediate filament protein found in the astrocytes, neuroglia end-feet and processes. Although neuroglia in normal rat retinas express little or no GFAP (Bignami & Dahl, 1979). Increased expression of GFAP in neuroglia in the retina and in astrocytes of the optic nerve is a well-known biomarker of retinal degeneration (Lewis & Fisher, 2003). In our study, GFAP expression increased in retina after trauma (Fig. 2e). Increased GFAP expression in the retina may be the result of an inflammatory response and tissue damage after Traumatic head injury. It is thought that the integrity of the cell-cell junction can be protected and the antioxidant effect as a result of the regulation of the

glial structure and the modification of the affected mullerian cells. We believe that *P. Fulgens* may be useful in preventing retinal damage resulting from traumatic head trauma, especially when used in clinics with adequate dosing and administration methods.

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RESUMEN: El trauma de cráneo es una de las principales causas de morbilidad y mortalidad. Como resultado de un traumatismo craneal, la retina puede sufrir diversos efectos bioquímicos, histológicos e inmunohistoquímicos. Veintiún ratas Sprague-Dawley fueron sometidas a lesión craneal traumática con un dispositivo, de caída de peso, usando un impacto de 300 g-1 m de peso-altura. Las ratas fueron divididas en tres grupos: grupo 1 (control), grupo 2 (traumatismo) y grupo 3 trauma + *Potentilla fulgens* (400 mg / kg / día, i. p.). Se usó agua destilada como vehículo en todos los grupos. Las ratas fueron decapitadas 5 días después de la inducción del trauma, y se evaluaron los efectos protectores de *P. Fulgens* mediante análisis histológicos, inmunohistoquímicos y bioquímicos. Es necesario realizar más estudios para evaluar los efectos neuroprotectores, dependientes del tiempo y la dosis, de *P. Fulgens*. Dependiendo si el trauma inhibe la apoptosis de las células fotorreceptoras, se estima que la disposición de las células ganglionares ayuda contra la degeneración de las conexiones neuronales. *P. Fulgens* ha demostrado ser efectivo para el tratamiento de los trastornos oculares inducidos por lesión cerebral traumática.

PALABRAS CLAVE: Lesión traumática; Retina; *Potentilla fulgens*; Rata.

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