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Immunoexpression of Vascular Endothelial Growth Factor, β-cell Lymphoma 2 and Cluster of Differentiation 68 in Cerebellar Tissue of Rats Treated with *Ganoderma lucidum*

Inmunoexpresión del Factor de Crecimiento Endotelial Vascular, Linfoma de Células β 2 y Grupo de Diferenciación 68 en Tejido Cerebeloso de Ratas Tratadas con *Ganoderma lucidum*

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SUMMARY: Traumatic brain injury (TBI) can potentially lead to hemorrhages in all areas of the skull, which can damage cells and nerve connections. This study aims to investigate the protective effects of *Ganoderma lucidum* polysaccharides (GLPS) as a antioxidant on cerebellar cell tissues after traumatic brain injury in rats. Sprague Dawley rats were subjected to TBI with a weight-drop device using 300 g-1m weight-height impact. The groups are consisted of control, trauma, and trauma+*Ganoderma lucidum* groups. At seven days post-brain injury, experimental rats were decapitated after intraperitoneal administration of ketamine HCL (0.15 ml/100 g body weight). Cereballar samples were taken for histological examination or determination of malondialdehyde (MDA) and glutathione (GSH) levels and myeloperoxidase (MPO) activity. Significant improvement was observed in cells and vascular structures of *Ganoderma lucidum* treated groups when compared to untreated groups. It is believed that *Ganoderma lucidum* may have an effect on the progression of traumatic brain injury. *Ganoderma lucidum* application may affect angiogenetic development in blood vessel endothelial cells, decrease inflammatory cell accumulation by affecting cytokine mechanism and may create apoptotic nerve cells and neuroprotective mechanism in glial cells.

KEY WORDS: VEGF; βcl-2; CD68, *Ganoderma lucidum*; Traumatic brain injury; Cerebellum; Histopathology; Rat.

INTRODUCTION

Traumatic brain injury is an important cause of death, memory loss and physical disability. After trauma, nerve fiber damage and cell loss occur. Direct cerebellar injury is much less common than supratentorial trauma (Tsai *et al*., 1980). Some of the classically identified consequences of direct traumatic injury to the cerebellum are hypotonia, ataxia, dysmetria, tremor, dysdiadochokinesis, and vertigo (Potts *et al*., 2009). Cells from various brain regions respond differently to mechanical injury and Purkinje neurons are most affected by cerebellar trauma (Slemmer *et al*., 2004).

Ganoderma lucidum is a medicinal mushroom and used in traditional Chinese medicine, with a very broad spectrum of biological activities and pharmacological functions (Potts *et al*.). *Ganoderma lucidum*, called "Lingzhi" in Chinese and "Reishi" in Japanese, is one of the most commonly used mushrooms by traditional Chinese medicine in Asia (Paterson *et al*., 2006). Ancient Chinese medical scholars suggested that *G. lucidum* could strengthen body resistance and consolidate the constitution of patients, ie, "Fuzheng Guben", which is one of the major principles in the therapeutics of traditional Chinese medicine (Cong & Lin, 1981). Nerve growth factor has potent biological activities such as promoting neuronal survival and neuritogenesis (D'Ambrosi *et al*., 2000). It is targeted as a potential therapeutic drug for the treatment of neurodegenerative disorders (Hefti & Weiner, 1986; Connor & Dragunow, 1998). However, nerve growth factor is unstable and is unable to cross blood-brain barrier because of its high molecular polypeptide (Granholm *et al*., 1998).

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Hence, the potential medicinal values of culinary and medicinal mushrooms have attracted intense interest in the search for pharmacological compounds that mimic the nerve growth factor activity in the prevention of neurodegenerative diseases.

G. lucidum is reportedly known to have anticancer, antitumor, antidiabetic, and anti-inflammatory effects (Harhaji Trajkovic´ *et al*., 2009; Weng *et al*., 2009; Huang *et al*., 2010). Oral administration of *Ganoderma lucidum* has been shown to significantly reduce both cerebral infarct area and neuronal apoptosis in the ischemic cortex (Zhou *et al*., 2012). Recent studies have demonstrated the neuroprotective effect of *Ganoderma lucidum* to reduce oxidative stress invitro (Zhao *et al*., 2005) to induce neuronal differentiation (Cheung *et al*., 2000) and to prevent the harmful effects of the exterminating toxin Ab in Alzheimer's disease in cultured rat neurons (Lai *et al*., 2008). Moreover, neuroprotective effects of *G. lucidum* have been demonstrated. Polysaccharides from *G. lucidum* protected rat cerebral cortical neurons from injury induced by hypoxia/reoxygenation ex-posure (Zhao *et al*., 2004) as well as reduced neurological deficits in rats after cerebral ischemic injury (Zhou *et al*., 2010). Furthermore, *G. lucidum* extract protected dopaminergic neurons from degeneration (Zhang *et al*., 2011). Altogether, *G. lucidum* has been postulated as a novel therapeutic approach in the prevention and treatment of neurodegenerative diseases such as stroke (Zhao *et al*., 2004; Zhou *et al*., 2010) Parkinson disease (Zhang *et al*., 2011) or Alzheimer disease (Zhou *et al*., 2010).

VEGF-induced blood vessel growth is essential for nervous tissue growth during embryonic development. This is demonstrated by the observation that loss of VEGF expression by central nervous system (CNS) neurons impairs vascularisation, curbs neuronal expansion and results in neuronal apoptosis in the developing brain (Haigh *et al*., 2003; Raab *et al*., 2004). The blood-brain barrier is a dynamically regulated physical barrier between the central nervous system and circulation consisting of endothelial cells that line cerebral microvessels. While the BBB sustains the unique chemical microenvironment critical for neuronal activity in the central nervous system, it also restricts access to therapeutic drugs. VEGF increases permeability of blood-brain barrier (BBB) by induction of synthesis and secretion of nitric oxide (NO) and activity of cGMP (Nag *et al*., 1997). Bcl-2 is an integral membrane protein that functions primarily as an inhibitor of apoptosis. Overexpression of Bcl-2 in both neuronal and hematopoietic cell lines is protective following growth factor withdrawal (Garcia *et al*., 1992; Allsopp *et al*., 1993; Batistatou *et al*., 1993). In vivo overexpression of BCL-2 during embryogenesis or postnatally can significantly reduce the extent of naturally occurring cell death as well as rescue many neurons from external injuries (e.g. axotomy or ischemia) or genetic lesions (Dubois-Dauphin *et al*., 1994; Farlie *et al*., 1995; Chen *et al*., 1996; de Bilbao & Dubois-Dauphin, 1996). Using a model of combined traumatic brain injury and hypoxemia, Clark *et al*. (1997) observed an up-regulation of Bcl-2 in cortical and hippocampal neurons that survived the traumatic injury. On the other hand, CD 68-positive microglial cells have frequently been observed in human fetal white matter (Andjelkovic *et al*., 1998; Rakic & Zecevic, 2003). In the aged rat brain there is an increase in CD68 + cells throughout the parenchyma in both grey and white matter and appearance of MHCII positive aggregates of cells in and adjacent to white matter (Perry *et al*., 1993). Similar changes have been observed in aged mice. These changes have been associated with an increased sensitivity to systemic inflammatory challenge with increased cytokine production and altered behavioural responses (Barrientos *et al*., 2006; Chen *et al*., 2008; Henry *et al*., 2009; Wynne *et al*., 2010).

The purpose of this study was to evaluate immunoexpressions of vascular endothelial growth factor, Bcl-2, CD68 and Western blot analysis in cerebellar tissues treated with *Ganoderma lucidum* after traumatic brain injury in rats.

MATERIAL AND METHOD

Animals and experimental design. 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 procedures performed in this experiment were approved by the Ethics Committee for the Treatment of Experimental Animals (Faculty of Medicine, University of Dicle, Turkey, Protocol Number: 2016/15). Male Sprague-Dawley rats (280-330 g) were maintained under 23±2°C and 12 h light/dark cycles with ad libitum access to standard pelleted food and water. A rectal probe was inserted, and the animals were positioned on a heating pad that maintained the body temperature at 37 °C. The widely used diffuse brain injury model described by Marmarou *et al*. (1994). Briefly, a trauma device which works by dropping a constant weight from a specific height through a tube was used. A weight of 300 g was dropped from a 1 m height, which can induce mild trauma, as shown by Ucar *et al*. (2006). The rats were divided into 3 groups as control, trauma and trauma+*Ganoderma lucidum* groups (20 ml/kg per day via gastric gavage). Thirty minutes after the trauma, rats were injected *Ganoderma lucidum* polysaccharides via gastric gavage for seven days in trauma+*Ganoderma lucidum* group (n=12). The rats of control (n=12) and trauma groups (n=12) were only administered 1.5 ml physiologic saline solution subcutaneously for 7 days. All rats at the end of experiment

were no difference in food/water consumption and body weight gain between experimental and control rats were observed. After seven days, all animals were sacrificed 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). Each group had 12 animals. All of them were utilized for biochemical parameters and the assessment of histological examination in each group.

Histological investigations. Tissue samples were homogenized with ice-cold 150 mM KCl for the determination of malondialdehyde and glutathione levels. The MDA levels were assayed for products of lipid peroxidation, and the results were expressed as nmol MDA/ g tissue (Sohrab *et al*., 2015). GSH level was determined by the spectrophotometric method, which was based on the use of Ellman's reagent, and the results were expressed as mmol glutathione/g tissue (Preston & Phillips, 2016). Myeloperoxidase activity in tissues was measured by a procedure similar to that described by Hillegass *et al*. (1990). Myeloperoxidase activity was expressed as U/g tissue. To evaluate the blood-brain barrier (BBB) integrity, Evans blue (EB) dye was used as a marker of albumin extravasation (Hakan *et al*., 2010). EB was expressed as mg/mg of cerebellar tissue against a standard curve. Cerebellar edema was evaluated by the drying-weighing method based on the measurement of the water content of the brain (Gumerlock *et al*., 1996). The percentage of water was calculated according to the following formula: %H2O = [(wet weight-dry weight)/wet weight] X 100. At the end of the experiment, all animals were anesthetized via the intraperitoneal administration of ketamine HCL (0.15 ml/100 g body weight). The cerebellum were dissected. For the histological examination, cerebellum tissues were fixed in 10 % formaldehyde solution, post fixed in 70 % alcohol, and embedded in paraffin wax. The sections were stained with H&E for histopathological examinations.

Immunohistochemical technique. Sections were brought to distilled water and washed in 3 x 5 min Phosphate Buffered Saline (PBS). Catalog number 10010023, Thermo Fischer Scientific Fremont, CA, USA. Antigen retrieval was done in microwave (Bosch®, 700 watt) for 3 min x 90 ºC. They were subjected to a heating process in a microwave oven at 700 watts in a citrate buffer (pH 6) solution for proteolysis. Sections were washed in 3 x 5 min PBS and incubated with hydrogen peroxide [K-40677109 ,64271 Hydrogen peroxide (H_2O_2) Dortmund+Germany, MERCK] (3 ml % 30 Hydrogen peroxide (H_2O_2) + 27 ml methanol) for 20 min. Sections were washed in 3 x 5 min PBS min and blocked with Ultra V Block (lot: PHL150128, Thermo Fischer, Fremont, CA, USA) for 8 min. After draining, primary antibodies were directly applied to sections distinctly (Vascular Endothelial Growht Factor (VEGF), 1:100, lot#MA5-12184, Thermo Fischer, Fremont, CA, USA; B cell lymphoma-2, 1:100, lot#MA5-11757, Thermo Fischer, Fremont, CA, USA; Bax, 1:100, lot#MA5-12184, Thermo Fischer, Fremont, CA, USA; Cluster of Differentiation 68 (CD68), 1:100, lot#MA5-13324, Thermo Fischer, Fremont, CA, USA) for 14 min. After washing with PBS, Streptavidin Peroxidase (lot: PHL150128, Thermo Fischer, Fremont, CA, USA) was applied to sections for 15 min. Sections were washed in 3x5 min PBS and Diaminobenzidine (DAB, Invitrogen, Carlsbad, lot: HD36221, Thermo Fischer, Fremont, CA, USA) were applied to sections up to 10 min. Slides showing reaction was stopped in PBS. Counter staining was done with Harris's Haematoxylin for 45 sec, dehydrated through ascending alcohol and cleared in xylene. Product Number: HHS32 SIGMA, Hematoxylin Solution, Harris Modified, Sigma-Aldrich, 3050 Spruce Street, Saint Louis, MO 63103, USA. Slides were mounted with Entellan® (lot: 107961, Sigma-Aldrich, St. Louis, MO, United States) and examined under Olympus BH-2 light microscopy.

Western Blotting

Cell lysis and protein quantification. The snap frozen cerebellum was grinded to a fine powder in a chilled mortar in the presence of liquid nitrogen. Immediately after grinding, 50 mg cerebellum powder was transferred into a microcentrifuge tube containing 250 µl RIPA lysis buffer (150 mM NaCl, 50 mM Tris, 1 mM EDTA, 1 % (v/v) Triton X-100, 1 % (w/v) sodium deoxycholate, 0.1 % (w/v) SDS, 0.2 % (w/v) sodium fluoride, 0.2 % (w/v) sodium orthovanadate and 1¥ protease inhibitor mixture (CompleteTM EDTA free; Roche Diagnostics) and incubated in ice for 1 h. After incubation, aliquots were snap-frozen in liquid nitrogen and stored -86 °C. All these steps were performed on ice to minimize protein degradation. Total cellular protein concentration was determined in triplicate using a BCA protein assay according to manufacturer's instructions (Pierce, Thermo scientific). The BCA assay was performed in a 96-well plate using MultiscanTM GO microplate from Thermo Scientific.

SDS-PAGE. All protein samples were resolved by 10 % (v/ v) polyacrylamide gel using a Mini Protean Tetra Cell apparatus system (Bio-Rad). The protein samples were prepared in 1¥ SDS loading buffer (2 % (w/v) SDS, 5 % (v/ v) glycerol, 0.01 % (w/v) bromophenol blue, 8 % (w/v) DTT, which was added just before heating) and heated at 95 °C for 5 min. The protein samples (20 µg) were then loaded on to the 10 % (v/v) polyacrylamide gel and electrophoresed at 200 V for 1 h in a SDS running buffer (2.4 mM Tris, 19.2 mM glycine, 0.01 % (w/v) SDS).

Membrane transfer and antibody staining. Separated proteins from the SDS-PAGE were transferred onto the PVDF membrane at 100 V for 1 h in transfer buffer (25 mM Tris, 192 mM glycine, 20 % (v/v) methanol, pH 8.3) using a Criterion Blotter Transfer System (Bio Rad). The membrane was then blocked with 5 % (w/v) skim milk powder in PBS-T (PBS+ 0.1 % (v/v) Tween-20) for 1 h at room temperature. After blocking, the membrane was probed with primary antibodies in 1: 1000 dilution (anti-Bcl-2, anti-VEGF and anti-CD68 obtained from Santa Cruz Biotechnology for 2 h at room temperature. The membrane was then washed four times over 30 min with PBS-T before probing with horseradish peroxidase-conjugated secondary antibodies in 1:10000 dilutions for 1 h at room temperature. The membrane was again washed four times over 30 min with PBS-T. The protein bands were visualized using ECL (Bio-Rad) according to manufacturer's instruction. The images were taken using ChemiDocTM MP (Bio-Rad).

Statistical analysis. Data analysis was performed using SPSS for windows, version 18 (SPSS, Inc., Chicago, IL, USA). All data were presented as mean ± 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

In our study, control, trauma and trauma+*Ganoderma lucidum* groups were compared in terms of biochemical datas. MPO activity, which is accepted as an indicator of neutrophil infiltration, was significantly higher in the cereballar tissues of traumatic rats than those of the control group ($p<0.001$). *Ganoderma lucidum* treatment significantly decreased cerebellar tissue MPO levels ($p<0.001$). Trauma caused a significant increase in the MDA levels ($p<0.001$) with a concomitant decrease in GSH levels ($p<0.001$). *Ganoderma lucidum* treatment significantly reduced the increase in MDA levels and restored GSH content at day 7. Figs. 1-3

Tissue EB content was significantly higher in the cereballar tissues of traumatic rats than those of the control group ($p<0.001$). *Ganoderma lucidum* treatment significantly decreased cerebellar tissue EB content ($p<0.001$). And, brain water content, which is accepted as an indicator of edema, was significantly higher in the cereballar tissues of traumatic rats than those of the control group ($p<0.001$). Results of biochemical analysis were shown in Table I.

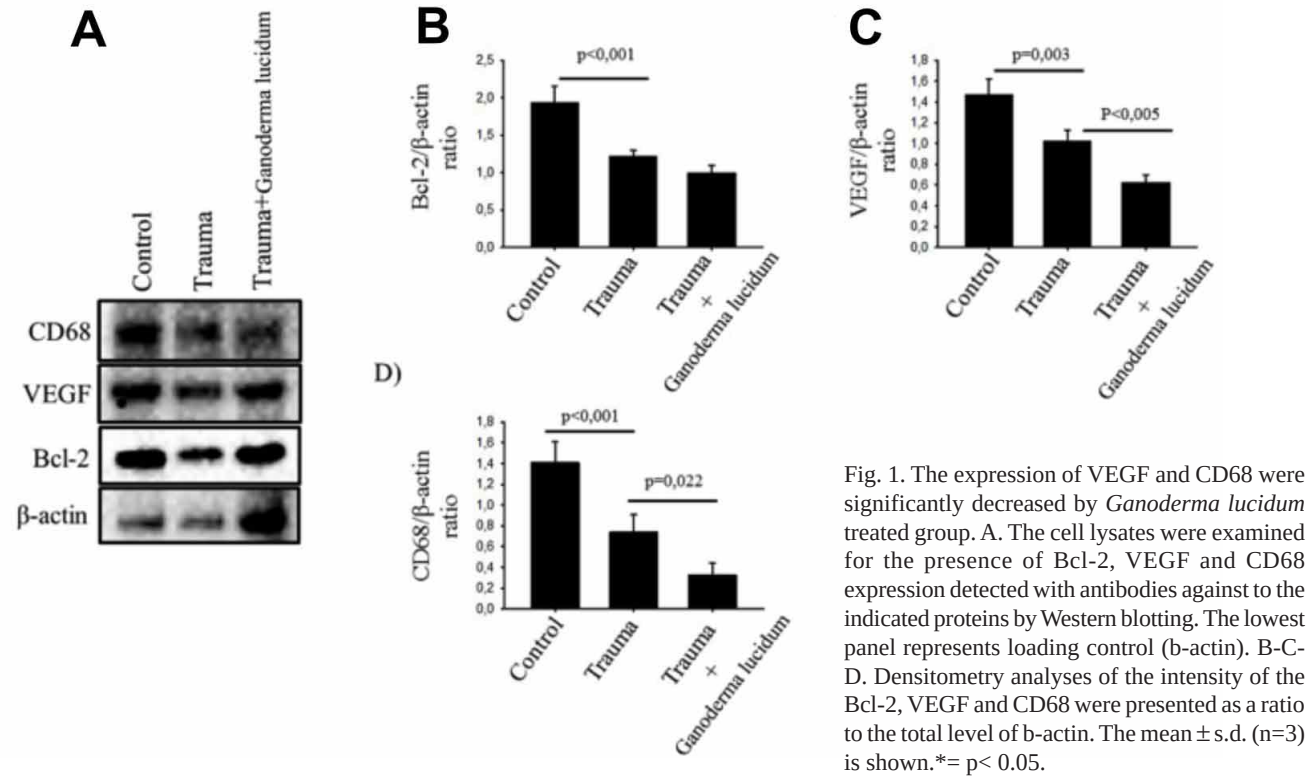

Fig. 1. The expression of VEGF and CD68 were significantly decreased by *Ganoderma lucidum* treated group. A. The cell lysates were examined for the presence of Bcl-2, VEGF and CD68 expression detected with antibodies against to the indicated proteins by Western blotting. The lowest panel represents loading control (b-actin). B-C-D. Densitometry analyses of the intensity of the Bcl-2, VEGF and CD68 were presented as a ratio to the total level of b-actin. The mean ± s.d. (n=3) is shown.*= $p< 0.05$.

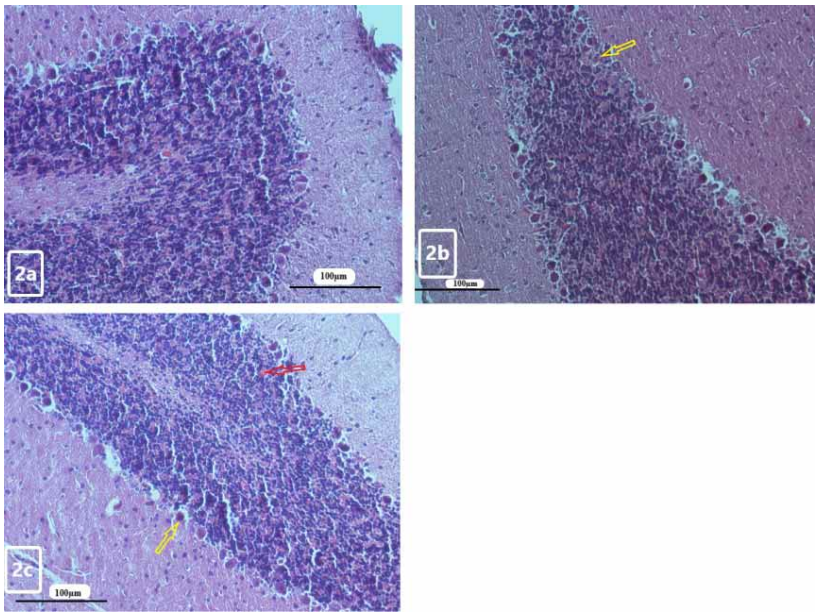

Fig. 2a-Control group. Normal appearance of the cerebellar tissue, H&E staining Bar 100 µm, 2b-Trauma group. Degeneration and vacularisation in some purkinje cells in the ganglion layer (yellow arrow) and hemorrhagie in small capillary vessels (red arrow), H&E staining, Bar 100 µm, 2c-Trauma+G.lucidum group. Small vacuolization and poor degeneration in purkinje cells (yellow arrow) was regularly seen towards the molecular layer of the extensions in the periphery. Weak hemorrhage in small veins in the granular layer (red arrow), the stratum granular and glial cells were observed as oval-shaped, H&E staining Bar 100 µm.

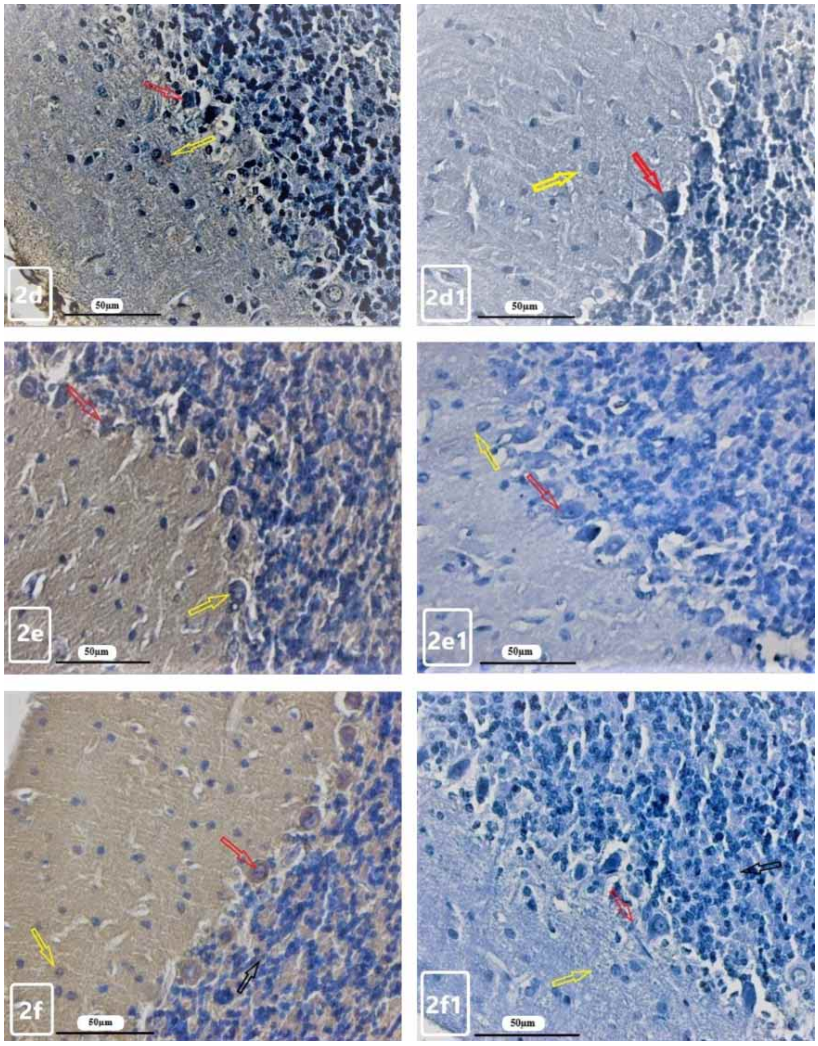

Fig. 2d-Control group. Weak CD68 expression in glia (yellow arrow) and Purkinje cells (red arrow) in the substantia grisea and substantia alba layer, CD68 immunostaining Bar 50 µm, 2d1-Control group. CD68 immunostaining negative slides, Bar 50 µm, 2e-Trauma group. Positive CD68 expression in glia (yellow arrow) and Purkinje cells (red arrow), CD68 immun-staining Bar 50 µm, 2e1-Trauma group. CD68 immunostaining negative slides, Bar 50 µm, 2f-Trauma+G.lucidum group. Weak CD68 expression in glia cells (yellow arrow), Purkinje cells (red arrow), and granular cells (black arrow), CD68 immun-staining Bar 50 µm, 2f1-Trauma+G.lucidum group. CD68 immunostaining negative slides, Bar 50 µm.

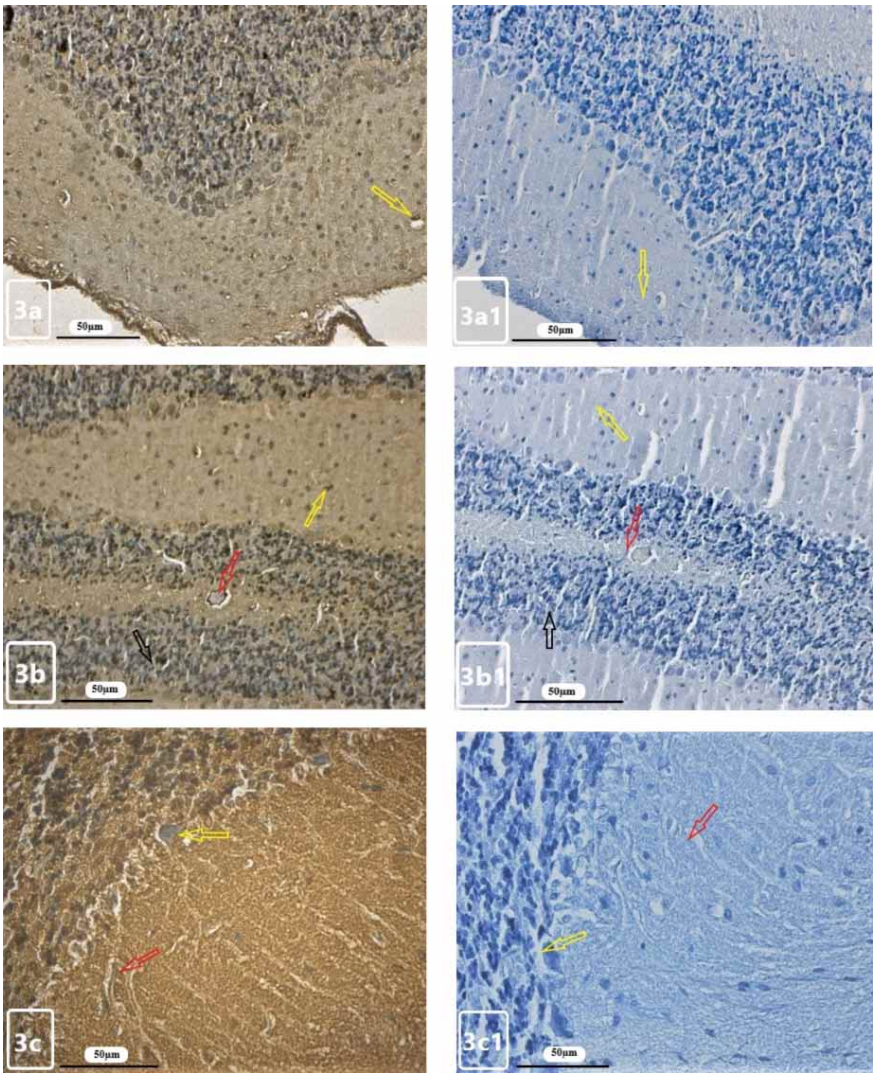

Fig. 3a-Control group. Positive VEGF expression in capillary vascular endothelial cells (yellow arrow), VEGF immunstaining Bar 50 µm, 3a1-Control group. VEGF immunostaining negative slides, Bar 50 µm, 3b-Trauma group. Positive VEGF expression in the glomerular areas (black arrow), glial (yellow arrow) and endothelial cells (red arrow) in the molecular layer, VEGF immunostaining Bar 50 µm, 3b1-Trauma group. VEGF immunostaining negative slides, Bar 50 µm, 3c-Trauma+G.lucidum group. Positive VEGF expression in small blood vessels (red arrow), and weak VEGF expression in purkinje cells (yellow arrow), VEGF immunostaining Bar 50 µm, 3c1-Trauma+G.lucidum group. VEGF immunostaining negative slides, Bar 50 µm, 3d-Control group. Negative Bcl-2 expression in Purkinje (red arrow) and glial cells (yellow arrow), Bcl-2 immunostaining Bar 50 µm, 3d1-Control group. Bcl-2 immunostaining negative slides, Bar 50 µm, 3e-Trauma group. Positive Bcl-2 expression in Purkinje cells (red arrow) and glomerular area (yellow arrow), Bcl-2 immunostaining Bar 50 µm, 3e1-Trauma group. Bcl-2 immunostaining negative slides, Bar 50 µm, 3f-Trauma+G.lucidum group. Reduction of Bcl2 expression intensities in some purkinje (red arrow) and granular cells (yellow arrow), Bcl-2 immunostaining Bar 50 µm, 3f1-Trauma+G.lucidum group. Bcl-2 immunostaining negative slides, Bar 50 µm.

Table I. Biochemical results relevant to the study groups.

	Control	Trauma	Trauma+ *G. lucidum*
MDA (nmol/g)	36.74±1.08	56.83±1.67***	$49.1\pm1.36^{+++,**}$*
GSH (µmol/g)	1.13±0.1	0.76±0.13***	$1.04\pm0.09^{+++}$**
MPO (U/g)	5.55±0.67	8.26±0.7***	$6.09\pm0.49^{+++}$*
Brain Water Content (%)	81.37±1.28	88.51±1.4***	$83.08\pm0.97^{+++}$***
Blood-Brain Barrier Permeability (mg/g)	6.17±0.79	9.18±0.72***	$6.57\pm0.52^{+++}$

Values are represented as mean ± SD. Each group consists of twelve rats. MDA (Malondialdehyde), GSH (Glutathione), MPO (Myeloperoxidase), *** p<0.001, versus control,** p<0.01, versus control ,* p<0.05, versus control ,+++p<0.001, versus trauma ,++p<0.01, versus trauma
+p<0.05, versus trauma

DISCUSSION

Ganoderma lucidum polysaccharides also has long been used as a traditional medicine for revival and a long life, whose hyphal body or the constituents is reported to have various physiological activities including antihyperglycemic (Zhang & Lin, 2004) immunomodulating (Zhu & Lin, 2005), antineoplastic (Gao *et al*., 2005), antiviral

(Liu *et al*., 2004), cholesterol-lowering (Hajjaj *et al*., 2005), and antioxidative effects (Wong *et al*., 2004). As *G. lucidum* also contains solid medium-degradation products, including water-soluble lignin caused by the hyphal bodies and hyphal autodigestion in addition to hyphal body components, its activity may differ from that of Reishi mushroom. The activities that have been reported include antihyperglycemic (Usui *et al*., 2007; Kamiuchi *et al*., 2010; Kawahara *et al*., 2011; Kamiuchi *et al*., 2014) , immunostimulating (Nakagawa *et al*., 1999), antineoplastic (Lu *et al*., 2003), antidepressive-like (Matsuzaki *et al*., 2013), and antihypertensive effects (Fukaya *et al*., 2015).

Polysaccharides, isolated from *G. lucidum* fruiting bodies, have antioxidant (Liu *et al*.) immunomodulatory (Bao *et al*., 2001) and antitumor properties (Cao & Lin, 2006). Moreover, polysaccharides were protective against cerebral ischemic injury (Zhou *et al*., 2010) and traumatic spinal cord injury in rats (Gokce *et al*., 2015). GLPS induced neuronal differentiation of pheochromocytoma cell cultures and protected PC12 neurons from apoptosis, by the Erk1/2 and the CREB signaling pathways. And, they reported that *Ganoderma lucidum* contains neuroactive compounds that can induce neural differentiation and prevent apoptosis of NGF-dependent neurons (Transforming growth factor) (Cheung *et al*.). *G. lucidum* extracts decreased inflammatory mediator production by activated microglia and protected dopaminergic neurons against inflammatory and oxidative damage (Zhang *et al*., 2011). Furthermore, *G. lucidum* spores preserved injured spinal motor neurons by modulating expression of proteins important for axonal regeneration (Zhang *et al*., 2006). These findings suggested that polysaccharides isolated from *G. lucidum* had both neuroprotective and antioxidant properties. Moreover, Sun *et al*. (2017) reported on the neuroprotective effects of GLPS against oxidative stress-induced apoptosis in cultured cerebellar granule cells.

G. lucidum extract reduced the expressions of proinflammatory and cytotoxic factors from the activated microglia, and effectively protected the dopaminergic neurons against inflammatory and oxidative damage (Huang *et al*., 2012) . After trauma, increased protein expression of VEGF causes increased blood vessel permeability.

Ganoderma lucidum administered post-traumatized VEGF is thought to help reduce the concentration of VEGF and reduce vascular permeability. Strong microglial activation is a common inflammatory response observed following injury to the cerebellum. Following either fluid percussion injury or controlled-cortical impact, activated microglia are organized perpendicular to the Purkinje cell layer (Fukuda *et al*., 1996; Mautes *et al*., 1996). After traumatic brain injury, Activated microglia Purkinje cell damage is reported to be a sensitive indicator and may act as a cell cleaner to remove debris and promote wound healing (Kim & de Vellis, 2005). CD68 expression,especially in the glomerular area of the granular layer, increased in the microglia cells after trimming. In accordance with this, there was an increase in VEGF expression in endothelial cells and inflammatory cells in enlarged blood vessels.

The amount of apoptosis necessary for physiological brain development is determined by the degree of myelinization and the water content of the brain (Smith *et al*., 1995). It has been suggested that apoptosis of oligodendrocytes after traumatic CNS injury may be a result of either the direct trauma or a secondary event due to loss of trophic support from the degenerating axons (Beattie *et al*., 1998). Aqueous extract of *Ganoderma lucidum* significantly attenuated Ab-induced synaptotoxicity and apoptosis by preserving the synaptic density protein called synaptophysin (Lai *et al*.). Further, a study by Wang *et al*. (2004) concluded that senescence-accelerated mice (strain SAMP8) given a diet supplemented with Ganoderma extract exhibited significantly lower brain amyloid and higher antioxidation activities such as superoxide dismutase, glutathione peroxidase (GPx), and glutathione reductase when compared with the control mice.

CONCLUSION

Ganoderma lucidum polysaccharides have protective effects against apoptosis and inflamations in neurons exposed to traumatic brain injury in rats. *Ganoderma lucidum* is thought to induce angiogenetic development in cerebellar tissue after traumatic brain injury, reducing inflammation and apoptotic changes in nerves and glial cells. Small vacuolization and poor degeneration in Purkinje cells was regularly seen towards the molecular layer of the extensions in the periphery. Weak hemorrhages in small veins were seen in the granular layer. The stratum granular cells and glial cells were observed as oval-shaped. Positive VEGF expression was seen in the glomerular areas, glial and endothelial cells in the trauma group, which suggest that VEGF may be derived from astrocytes and neurons. In addition, we determined that weak VEGF expression was seen in purkinje cells, weak CD68 expression was seeen in microglia, Purkinje and granular cells, and reduction of Bcl2 expression intensities was seen in Purkinje and granular cells in the treated *Ganoderma lucidum* group. These histopathological findings suggested that *Ganoderma lucidum* polysaccharides may play a certain role for expression of apoptosis-associated proteins and have

significant neuroprotective effects. Although apoptosis ascribed to oxidative stress and inflammatory processes is believed to play a major role, *Ganoderma lucidum* polysaccharides has been shown to suppress necrosis, protect neural structures, as well as neuronal apoptosis, indicating that further studies are needed concerning the mechanism of activity because the cereballar-protective effect of *Ganoderma lucidum* polysaccharides cannot be solely explained by histopathological findings.

ÖZEVREN, H.; IRTEGÜN, S.; EKINGEN, A.; TUNCER, M. C.; GÖKALP ÖZKORKMAZ; DEVECI, E. & DEVECI, S. Inmunoexpresión del factor de crecimiento endotelial vascular, linfoma de células β 2 y grupo de diferenciación 68 en tejido cerebeloso de ratas tratadas con *Ganoderma lucidum*. *Int. J. Morphol., 36(4)*:1453-1462, 2018.

RESUMEN: La lesión cerebral traumática (LCT) puede provocar hemorragias en todas las áreas del cráneo, lo que puede dañar las células y las conexiones nerviosas. Este estudio tuvo como objetivo investigar los efectos protectores de los polisacáridos de *Ganoderma lucidum* (GLPS) como antioxidante en los tejidos de las células del cerebelo después de la lesión cerebral traumática en ratas. Ratas Sprague Dawley fueron sometidas a TBI con un dispositivo de caída de peso usando un impacto de peso de 300 g-1 m. Se formaron los siguientes grupos: control, trauma y trauma + *Ganoderma lucidum*. Siete días después de la lesión cerebral, las ratas experimentales fueron decapitadas después de la administración intraperitoneal de ketamina HCL (0,15 ml / 100 g de peso corporal). Se tomaron muestras cerebrales para el examen histológico y para la determinación de niveles de malondialdehído (MDA) y glutatión (GSH) y actividad de mieloperoxidasa (MPO). Se observó una mejora significativa en las células y las estructuras vasculares de los grupos tratados con *Ganoderma lucidum* en comparación con los grupos no tratados. Durante el estudio se observó que *Ganoderma lucidum* puede tener un efecto sobre la progresión de la lesión cerebral traumática. La aplicación de *Ganoderma lucidum* puede afectar el desarrollo angiogénico en las células endoteliales de los vasos sanguíneos, disminuir la acumulación de células inflamatorias al afectar el mecanismo de las citocinas y puede crear células nerviosas apoptóticas y un mecanismo neuroprotector en las células gliales.

PALABRAS CLAVE: VEGF; Bcl-2; CD68, *Ganoderma lucidum*; **Lesión cerebral traumática; Cerebelo; Histopatología; Rata.**

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