Original Research

ANNALS OF MEDICINE & SURGERY

OPEN

Effect of the olive leaf extract in chronic spinal cord injury model: an experimental research

Iqbal P.A. Nasution, MD[a,*], Sabri Ibrahim, MD, PhD[b], Wibi Riawan, MD, PhD[c]

Introduction: Posttraumatic myelopathy is defined as a spinal cord injury (SCI) that results in varying degrees of motor and sensory deficits. The degree of 'secondary damage,' which is caused by a variety of cellular, molecular, and biochemical cascades is linked to the outcome of SCI. According to research, the beneficial effects of oleuropein and its derivatives have been linked to radical scavenging/antioxidant actions and anti-inflammatory effects.

Materials and Methods: This study was divided into six groups: control negative (sham-operated) group, control positive 1 and 2 (early chronic and chronic), treatment groups 1, 2, and 3 (prophylactic, concomitant, and late). Olive leaf extract (OLE) given dose was 350 mg/kg body weight. Blood was taken from the left corotic artery before the animals were terminated, seromarker assessment, enzyme-linked immunosorbent assay of IL-6, TNF-α, brain-derived neurotrophic factor (BDNF), and assessment of functional motoric outcome before the animal was terminated.

Results: Chronic spinal cord compression increased serum levels of IL-6, TNF-α, and decreased serum level of BDNF. OLE 350 mg/kg body weight decreased serum levels of IL-6, TNF-α and increased functional motoric outcome, especially in prophylactic and concomitant therapy.

Discussion: These findings indicate that OLE may be effective in protecting chronic SCI model.

Conclusion: Oleuropein has a potential effect to reduce the IL-6 and TNF-α in rabbit model of SCI, and the BDNF value risen after the administration of Oleuropein.

Keywords: chronic spinal cord injury, neuroprotective, olive leaf extract

Background

Posttraumatic myelopathy is a typical side effect after a SCI. The outlook is often bleak, with significant financial consequences. Posttraumatic myelopathy is defined as a SCI that results in varying degrees of motor and sensory deficits, with a yearly prevalence of around 12 000 cases in the USA[1]. Compression of the spinal cord results in ischemia and infarction, as well as biochemical and pathological changes in the spinal cord, resulting in posttraumatic myelopathy[2]. Cord cysts, syringomyelia, and ascending cystic cord degeneration are all associated with posttraumatic myelopathy, according to common wisdom. Although there are other causes of posttraumatic myelopathy, such as spinal instability, bone fragments that compress or pinch the spinal cord during trauma due to spinal canal stenosis, loculated subarachnoid cysts with cord compression, cord atrophy, and microcystic spinal, it can also take the form of loculated subarachnoid cysts with cord compression, cord atrophy, and microcystic spinal. Myelopathy with gliosis of the spinal cord and posttraumatic myelomalacia syndrome[3].

The degree of 'secondary damage,' which is caused by a variety of cellular, molecular, and biochemical cascades involving calcium ion influx, oxidative stress, inflammation, autoimmune response, vascular events, and apoptosis, is linked to the outcome of SCI. Because secondary injury appears to be responsive to pharmacological therapies, several studies have focused on modulating secondary damage pathways, including the use of antioxidant and anti-inflammatory medications[4]. Compression of the spinal cord causes neuroinflammation, which begins with damage to the blood–spinal-cord barrier, is followed by an immune response (expression of various cytokines, such as IL-1, IL-6, IL-12, IFN-γ, TNF-α), which causes reactive astrocyte cells (proliferation and scar formation), then activates Fas and caspase, resulting in apoptosis and necrosis of axons[5–7].

HIGHLIGHTS

- Olive leaf extract (OLE): Olives contain around 30 distinct phenol compounds, including oleuropein and oleocanthal.
- Chronic spinal cord injury (SCI): Compression of the spinal cord results in ischemia and infarction, as well as biochemical and pathological changes in the spinal cord, resulting in posttraumatic myelopathy.

Departments of [a]Surgery, [b]Neurosurgery, Faculty of Medicine, University of Sumatera Utara, Medan, Sumatera Utara and [c]Department of Biochemistry and Molecular Biology, Medical Faculty, Brawijaya University, Malang, Indonesia

This manuscript has been peer reviewed.

Sponsorships or competing interests that may be relevant to content are disclosed at the end of this article.

**Corresponding author. Address: Department of Surgery, Faculty of Medicine, Universitas Sumatera Utara, Sumatera Utara 20155, Indonesia. Tel.: +6261-8360143. E-mail address: pyloromyotomy@gmail.com (I.P.A. Nasution).*

Annals of Medicine & Surgery (2023) 85:365–372

Received 13 September 2022; Accepted 27 November 2022

Published online 9 March 2023

http://dx.doi.org/10.1097/MS9.0000000000000085

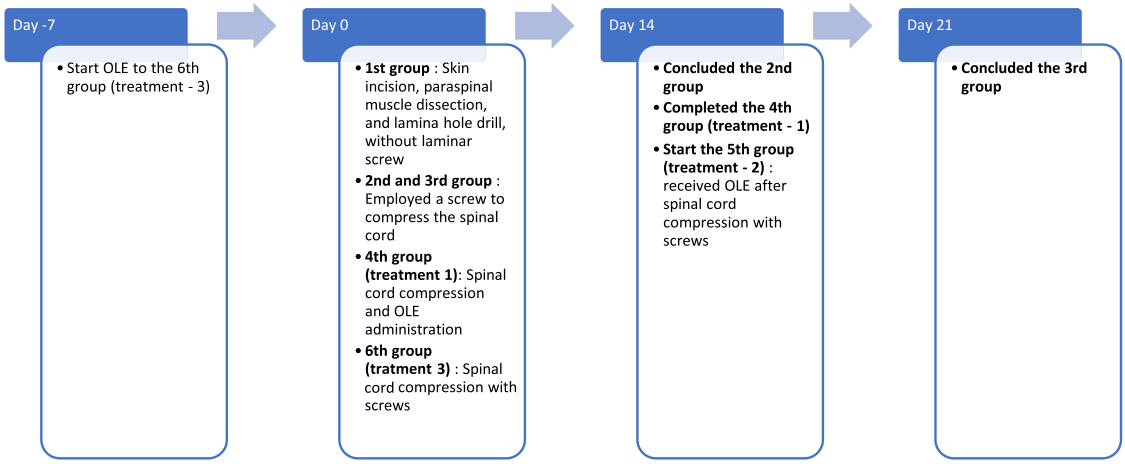

Figure 1. Experimental steps from day – 7 until day 14. OLE, olive leaf extract.

A 10-year prospective randomized study found no significant difference in outcomes or survival between conservative and surgical treatment in patients with mild and severe cervical spondylotic myelopathy (CSM). In recent years, neuroactive medications have shown promise in the treatment of CSM. Estrogens have been discovered to inhibit caspase-3, which reduces glutamate-induced apoptosis in neuronal cells. Tamoxifen, an estrogen receptor blocker, has been used to treat SCI after research showed that it can reduce reactive oxygen species and lipid peroxidation in the aftermath of ischemia/hypoxia. Riluzole was proven to reduce neuropathic pain in the CSM rodent model. Other antioxidants including pyrrolidine dithiocarbamate and vitamin E have also been shown to protect oligodendrocytes against apoptosis[8].

Sterols, carotenes, triterpenic alcohols, and phenolic compounds are among the 200 chemical components found in olives. Olives contain around 30 distinct phenol compounds, including oleuropein and oleocanthal, to name a few[4,9–14]. The hydrolysis of oleuropein, on the other hand, produces additional phenolics such hydroxytyrosol and tyrosol. Anti-inflammatory, antioxidant, skin-protecting, antiaging, antiviral, antimicrobial, anticancer, and antiatherogenic[15] characteristics are all found in olive polyphenols. According to research, the beneficial effects of oleuropein and its derivatives, such as hydroxytyrosol, have been linked to a variety of biological activities, including free radical scavenging/antioxidant actions and anti-inflammatory effects. In animal experiments, olive phenols were shown to have some protective effects against brain hypoxia–reoxygenation, cerebral ischemia, and brain damage after hypoxia–reoxygenation[16].

Despite some experimental evidence for olive phenolics' neuroprotective qualities in brain trauma, no research has been conducted to test if they protect against chronic SCI. We investigated the effects of olive polyphenol, a dietary antioxidant–anti-inflammatory component contained in olive leaves, on proinflammatory cytokine IL-6, TNF-α, neurotrophic factor synthesis [brain-derived neurotrophic factor (BDNF)], and functional motoric outcome in an experimental chronic SCI model. To undertake conservative therapy without decompression, we constructed a model of a moderate myelopathy condition. The researchers conducted two exploratory tests before developing animal models for this study, and the findings are consistent with posttraumatic myelopathy[17,18].

Materials and methods

Animal criteria

New Zealand's white rabbits In this study, males aged 12 weeks and weighing 2.6–3.0 kg (average: 2.9 kg) were used. Animals are fed and given water in a typical laboratory. With a 12-hour light–dark cycle, the room temperature is around 16–20°C.

Extraction process

In this study, OLE from Shaanxi Yongyuan BioTech Co. Ltd was used. The extract was suspended in distilled water and given to the animals via oral gavage in a 4 ml solution in the morning. It contains 40% oleuropein and has a dose of 350 mg/kg body

Table 1

Study group classification based on intervention

Group	Treatment
First group/negative control ($n=5$)	Skin incision, paraspinal muscle dissection, and lamina hole drill, without laminar screw
Second group/positive control-1 ($n=5$)	Employed a screw to compress the spinal cord and concluded on day 14
Third group/positive control-2 ($n=5$)	Employed a screw to compress the spinal cord and concluded on day 21
Fourth group/treatment-1 ($n=5$)	Spinal cord compression and OLE administration. Completed on day 14
Fifth group/treatment-2 ($n=5$)	Start on day 14: received OLE after spinal cord compression with screws
Sixth group/treatment-3 ($n=5$)	Given 7 days before spinal cord compression with screws

OLE, olive leaf extract.

Table 2

Motoric evaluation using modified Tarlov classification

Grade	Motor characteristics
0	Unable to have voluntary movements
1	Perceptible movements at join, the hindlimbs follow
2	Good movements at joins, but unable to stand up
3	Can stand up and walk, but unable to start running quickly
4	Normal

weight or 140 mg oleuropein. The dose stated has been converted to rabbit levels based on previous rat trials[4,9,19–21].

Experimental design

Thirty rabbits were divided into six groups for this experiment. The first group ($n = 5$) received a skin incision, paraspinal muscle dissection, and lamina hole drill, but no laminar screw was put. Positive control-1 ($n = 5$) was the second group, which employed a screw to compress the spinal cord and concluded on day 14. Positive control-2 ($n = 5$) was the third group, which employed a screw to compress the spinal cord and concluded on day 21. Treatment-1, which included spinal cord compression and OLE administration, was completed on day 14 in the fourth group ($n = 5$). After 14 days, the fifth group ($n = 5$) received OLE after spinal cord compression with screws. The study ended on day 21. Treatment-3, given 7 days before spinal cord compression with screws, was given to the sixth group ($n = 5$) (Fig. 1).

Surgical procedure

The rabbit was anesthetized by using 50 mg/kg of ketamine hydrochloride (Pfizer) and 10 mg/kg of Xylazine (Bayer), with cefazolin 50 mg/kg as profilactic[22]. Rabbit was in prone position, shaved in the posterior cervical area, disinfected with 10% betadine, and sterilized with cloth cover. We performed incision at C4–C6 posterior midline and small retractors was used. Palpation of spinous

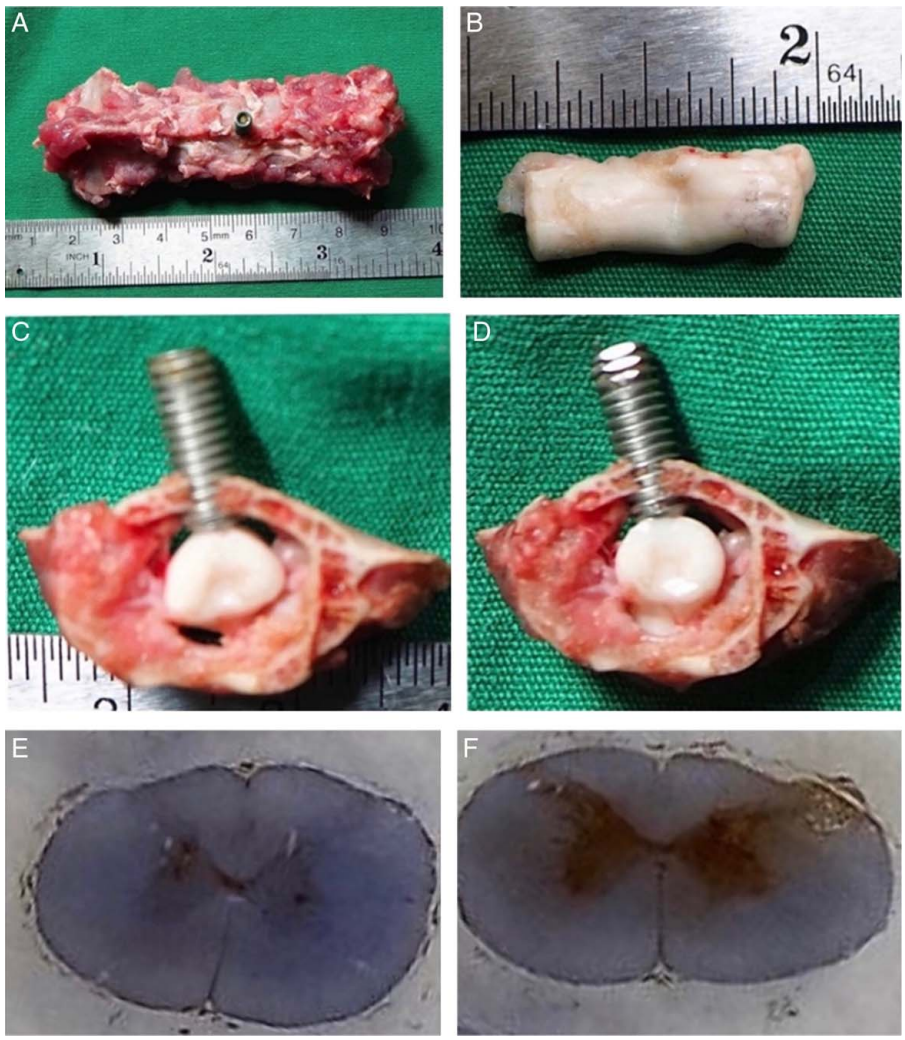

Figure 2. (A) C1–C7 rabbit cervical spine tissue with screw in midline lamina C5. (B) Spinal cord tissue at C4–C6 level, looks concave at screw compression area. (C) Cervical-5 with spinal cord compression screw day 14 (1 mm). (D) Cervical-5 with spinal cord compression screw day 21 (1.5 mm). (E) spinal cord sample from negative control, axial section. (F) Sample spinal cord from control positive-2, on compression area, spinal cord showed flattened on anterior–posterior, referring to chronic compression. Histological evaluation with hematoxylin and eosin showed lesion typical of chronic myelopathy, ischemic changes, and anterior horn alteration.

Table 3

Comparison of IL-6 levels between groups

	IL-6 level	
	ELISA (mean ± SD)	*P*-value
Control negative	445.81 ± 61.39	< 0.001
Control positive-1	578.09 ± 199.86	
Control positive-2	451.38 ± 52.08	
Treatment-1	370.03 ± 192.32	
Treatment-2	336.27 ± 50.04	
Treatment-3	270.36 ± 35.35	

ELISA, enzyme-linked immunosorbent assay; IL-6, interleukin-6.
One-way analysis of variance test, *P*-value significant if $P < 0.05$.

Table 4

Post-hoc analysis of IL-6 levels

	IL-6	
Groups	ELISA (Δ)	*P*-value
CN vs. C + 1	248.72	< 0.001
CN vs. C + 2	330.36	< 0.001
C + 1 vs. T1	− 470.90	< 0.001
C + 2 vs. T2	− 343.81	< 0.001
C + 2 vs. T3	− 500.00	< 0.001

C + 1, control positive-1; C + 2, control positive-2; CN, control negative; ELISA, enzyme-linked immunosorbent assay; IL-6, interleukin-6; Δ, mean difference; T-1, treatment-1; T-2: treatment-2; T-3: treatment-3.

processes, C5 paraspinal muscle was dissected and the lamina was identified. One hole is made in the lamina C5 at midline position using a high-speed diamond drill bur (3 mm in diameter) until it penetrates the lamina (2 mm thick lamina), the burr hole is tapered at 4 mm, then the lamina hole is inserted into a screw (stainless steel) with a diameter of 4 mm and a length of 10 mm through the lamina. On the first day, the compression is given 0.5 mm (by turning the screw 180°), on the seventh day, the screw is turned 180° again (total compression is 1 mm), on the 14th day the screw was rotated 180° (the total compression is 1.5 mm). After that installation, the skin was sutured. The position of the screw head is 0.5 cm below the skin, easily felt, so that in the second and third procedures, it is enough to open one skin suture and the screw is turned, the repeated procedure is carried out by sterilization, and the same anesthetic method.

Motor function evaluation

Motor function was evaluated by using the modification of Tarlov's classification[22] (Table 1). Evaluation was made before and immediately after the surgery and before animal termination (Table 2).

Enzyme-linked immunosorbent assay protocol

Blood was taken from the left carotid artery (before animal termination). After collection of the whole blood, blood sample was left undisturbed at room temperature for 15–30 min. The clot was removed by centrifuging at 1000–2000*g* for 10 min. The plasma then was maintained at – 20°C. Blood and plasma levels of IL-6, TNF-α, and BDNF were determined using a commercially available Sandwich ELISA System (Cusabio). TNF-α kit used was ELISA kit (Bender MedSystems): Rabbit TNF-α Platinum ELISA cat#BMS622. IL-6 kit used was ELISA kit (BioLegend): Legend Max Rabbit IL-6 ELISA Kit Cat#437107. BDNF kit used was BDNF Kit (BoosterBio): Rabbit BDNF ELISA Kit PicoKine Kit cat#EK0308.

Statistical analysis

Statistical analysis was performed using SPSS, Version 21 for Windows (SPSS Inc., Chicago, IL, USA). To test the significance of differences of the variable expression between the two experimental groups, we performed analysis of variance (ANOVA) tests. The significance level was defined as *P*-value less than 0.05.

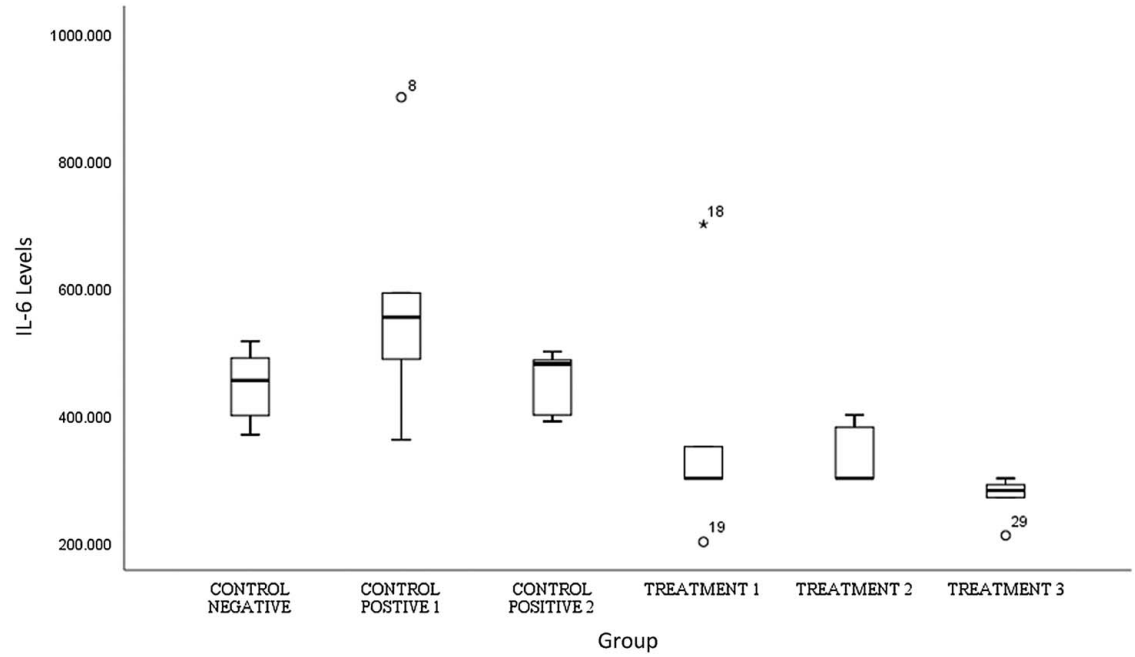

Figure 3. Graphic of serum interleukin-6 (IL-6) levels based on enzyme-linked immunosorbent assay examination.

Table 5

Comparison of TNF-α levels between groups

	TNF-α levels	
Groups	**ELISA (mean ± SD)**	***P*-value**
Control negative	109.80 ± 40.51	< 0.001
Control positive-1	281.20 ± 36.46	
Control positive-2	515.00 ± 109.84	
Treatment-1	127.20 ± 46.33	
Treatment-2	238.40 ± 46.63	
Treatment-3	130.40 ± 29.04	

ELISA, enzyme-linked immunosorbent assay; TNF-α, tumor necrosis factor α.
One-way analysis of variance test, *P*-value significant if $P < 0.05$.

Table 6

Post-hoc analysis of TNF-α levels

	TNF-α	
Groups	**ELISA (Δ)**	***P*-value**
CN vs. C + 1	− 171.40	0.001
CN vs. C + 2	− 405.20	< 0.001
C + 1 vs. T1	154.00	0.005
C + 2 vs. T2	276.60	< 0.001
C + 2 vs. T3	384.60	< 0.001

C + 1, control positive-1; C + 2, control positive-2; CN, control negative; ELISA, enzyme-linked immunosorbent assay; Δ, mean difference; T-1, treatment-1; T-2: treatment-2; T-3: treatment-3; TNF-α, tumor necrosis factor α.

Results

Animal model evaluation

A one-way homogenity ANOVA test was done and revealed that there was no significant differences between body weight before and after therapy ($P > 0.05$). This demonstrates that the animal body weight data is consistent, so that body weight is not a confounding variable that can alter the dependent variable.

After compression, clinical examination of experimental animals revealed no indications or symptoms of acute SCI. Mice with spinal cord compression experienced a gradual decline in motoric function until day 21. Control negative group '4,' control positive-1 '3,' control positive-2 group '2,' treatment-1 group '4,' treatment-2 group '2,', and treatment-3 group '3,' have similar motoric function before termination. The treatment-1 and treatment-3 groups showed a one-point improvement in motor performance, while the treatment-2 group showed no improvement.

Spinal cord specimen evaluation

In the area of compression, the spinal cord was seen flattened in the anterior–posterior direction indicating chronic compression, no signs of acute trauma was seen in the spinal cord tissue such as; intramedullary hemorrhagic, intramedullary contusion, and spinal cord laceration (Fig. 2).

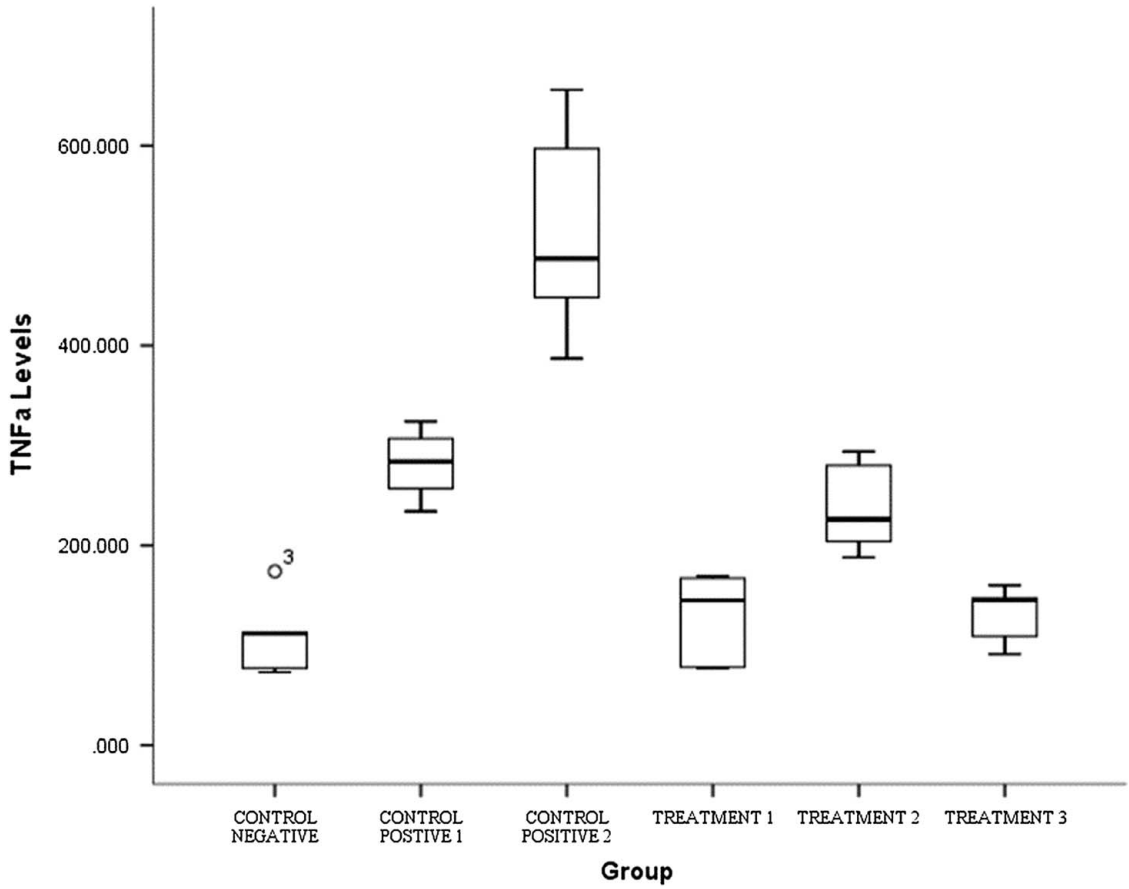

Figure 4. Graphic of serum tumor necrosis factor-α (TNF-α) levels based on enzyme-linked immunosorbent assay examination.

Table 7

Comparison of BDNF Levels between groups

	BDNF	
Groups	**ELISA (mean ± SD)**	***P*-value**
Control negative	114.75 ± 69.08	< 0.001
Control positive-1	32.87 ± 15.76	
Control positive-2	33.25 ± 17.43	
Treatment-1	244.25 ± 39.07	
Treatment-2	123.75 ± 19.07	
Treatment-3	339.12 ± 80.00	

BDNF, brain-derived neurotrophic factor; ELISA, enzyme-linked immunosorbent assay.
One-way analysis of variance test, *P*-value significant if $P < 0.05$.

Table 8

Post-hoc analysis of BDNF levels

	BDNF	
Groups	**ELISA (Δ)**	***P*-value**
CN vs. C + 1	81.87	0.180
CN vs. C + 2	81.50	0.185
C + 1 vs. T1	− 211.37	< 0.001
C + 2 vs. T2	− 90.50	0.092
C + 2 vs. T3	− 305.87	< 0.001

BDNF, brain-derived neurotrophic factor; C + 1, control positive-1; C + 2, control positive-2; CN, control negative; Δ, mean difference; ELISA, enzyme-linked immunosorbent assay; T-1: treatment-1; T-2: treatment-2; T-3: treatment-3

Interleukin-6

By using the one-way ANOVA test (Table 3) it can be seen that the treatment group 1 has a significant value with *P*-value (< 0.001). The post-hoc analysis test in Table 4, it can be seen that all group had a significant value on the ELISA examination ($P < 0.001$). The number can be seen in Figure 3.

Tunor necrosis factor-α

By using the one-way ANOVA test, in Table 5, it can be seen that the positive control group has a significant value against the other groups with *P*-value (< 0.001). Post-hoc analysis test in Table 6 shows that the significant value in the negative control group with positive control-2 ($P < 0.001$), positive control-2 with treatment-2 ($P < 0.001$), and treatment-2 with treatment-3 ($P < 0.001$), the graphic can be shown in Figure 4.

Brain-derived neurotrophic factor

By using one-way ANOVA test (Table 7) the result shows that all groups have a significant difference with *P*-value (< 0.001). Based on Table 8, the positive control group 1 compared with treatment-1 and the treatment group 2 compared with treatment-3 had a significant value based on ELISA with $P < 0.001$, based on Figure 5.

Discussion

According to this study, chronic spinal cord compression raises serum levels of IL-6 and TNF-α, while decreases BDNF. Oral OLE diet delivery reduced IL-6 and TNF-α levels in the blood, boosted BDNF levels, and improved functional motor outcomes, especially in early treatment (prophylactic and concomitant).

Three mechanisms are aided by the tropomycin kinase B receptor on BDNF. The first is through the PI3K/Akt pathway,

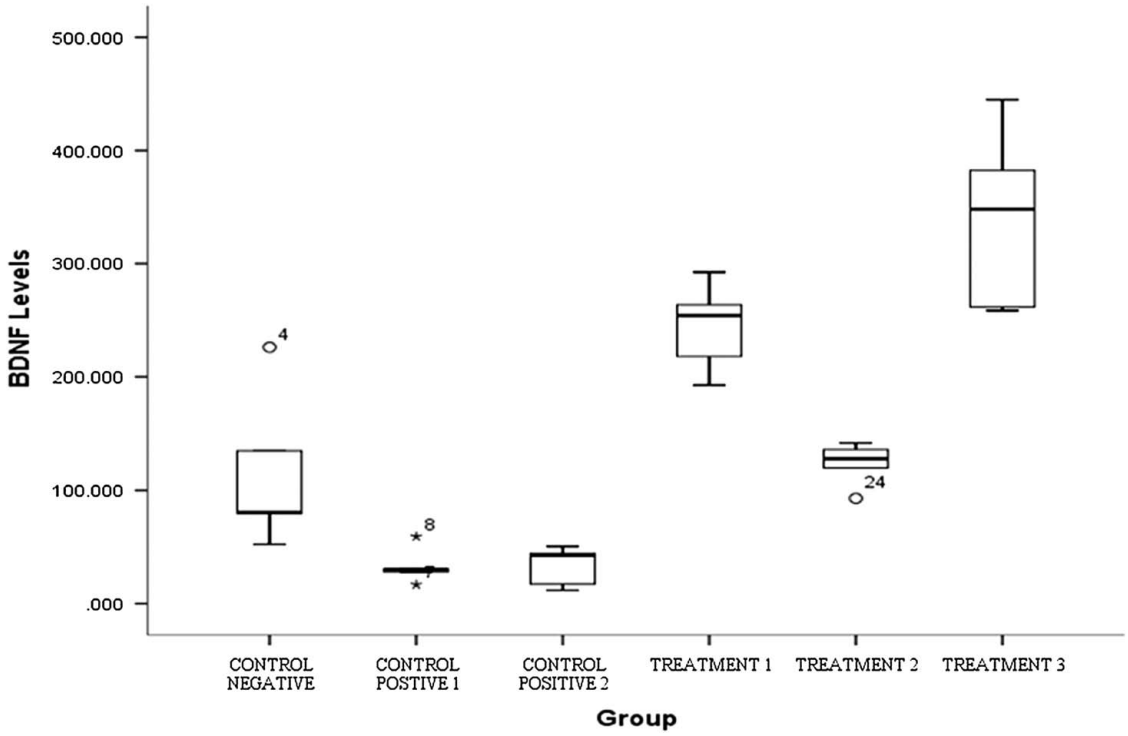

Figure 5. Graph of serum brain-derived neurotrophic factor (BDNF) levels based on enzyme-linked immunosorbent assay examination.

which is involved in cell survival; the second is through the phospholipase C pathway, which activates protein kinase C and CAMK, which is involved in neuroplasticity; and the third pathway is through Ras, which activates extracellular signal-regulated kinase, which is involved in plasticity, survival, and growth[23]. In SCI model research, oleuropein therapy increased the neurotrophic factor glial cell–derived neurotrophic factor[24]. Human investigations have shown that olive phenol increases BDNF levels and improves memory function[25]. In a rat model, eating an olive oil-rich diet during pregnancy and lactation significantly increased brain BDNF levels[26].

Increased levels/expression of IL-6 in the acute phase post-brain damage are neuroprotective and regenerative for neurons healing, but a continuous increase in the chronic phase produces poor prognosis of brain injury outcome, according to a recent literature review research[27].

Therapeutic oleuropein lowered TNF-α expression and altered the inflammatory response after spinal cord damage in rats with SCI[28]. Another study found that treating SCI patients with oleuropein aglycone reduced histological damage, improved motor recovery, and boosted levels of glial cell–derived neurotrophic factor[24].

Olive polyphenols reduced TNF-α and IL-6 levels and improved functional results in a rabbit model of SCI, according to a literature review[4]. In vitro, the olive phenol oleocanthal inhibited lipopolysaccharide-induced inflammation, lowering IL-6 and TNF-α. A review of the literature indicated that polyphenols from extra virgin olive oil reduced IL-6 and TNF-α levels[29,30].

The biomarker used were all beneficial in this study as the marker of chronic spinal myelopathy. The other marker may be used to identify myelopathy. The weakness of this study is that a bigger sample will need to be used for another type of experiment.

Conclusion

Oleuropein has a potential effect to reduce the IL-6 and TNF-α in a rabbit model of SCI, and the BDNF value increase after the administration of oleuropein.

Ethics approval and consent to participate

Institutional review board approval was obtained from the University of Sumatera Utara Ethics Committee for this study.

Sources of funding

None.

Authors' contribution

I.P.AN., S.I. and W.R.: creation to editing the manuscript.

Conflicts of interest disclosure

The authors declare that they have no financial conflict of interest with regard to the content of this report.

Research registration unique identifying number (UIN)

Not applicable.

Guarantor

Iqbal P.A. Nasution.

References

[1] Angeloni C, Malaguti M, Barbalace MC, *et al*. Bioactivity of olive oil phenols in neuroprotection. Int J Mol Sci 2017;18:2230.
[2] Anwar MA, Al Shehabi TS, Eid AH. Inflammogenesis of secondary spinal cord injury. Front Cell Neurosci 2016;10:98.
[3] Barbaro B, Toietta G, Maggio R, *et al*. Effects of the olive-derived polyphenol oleuropein on human health. Int J Mol Sci 2014;15:18508–24.
[4] Bucciantini M, Leri M, Nardiello P, *et al*. Olive polyphenols: antioxidant and anti-inflammatory properties. Antioxidants 2021;10:1044.
[5] De Smet E, Vanhoenacker FM, Parizel PM. Traumatic myelopathy: current concepts in imaging. Semin Musculoskelet Radiol 2014;18: 318–31.
[6] Desimone A, Laliberte AM, Fehlings MG. The influence of ApoE4 on the clinical outcomes and pathophysiology of degenerative cervical myelopathy. JCI Insight 2021;6:e149227.
[7] Falcone S, Quencer RM, Green BA, *et al*. Progressive posttraumatic myelomalacic myelopathy: imaging and clinical features. Am J Neuroradio/ 1994;15:747–54.
[8] Gao Y, Li X, Xu R, *et al*. Oleuropein improved post cerebral stroke cognitive function by promoting histone acetylation and phosphorylation of cAMP response element-binding protein in MCAO rats. Dose Response 2020;18:1559325820950102.
[9] Garraway SM, Huie JR. Spinal plasticity and behavior: BDNF-induced neuromodulation in uninjured and injured spinal cord. Neural Plast 2016;2016:9857201.
[10] Goren L, Zhang G, Kaushik S, *et al*. (–)-Oleocanthal and (–)-oleocanthal-rich olive oils induce lysosomal membrane permeabilization in cancer cells. PLoS One 2019;14:e0216024.
[11] Ibrahim S, Riawan W. Progressive spinal cord compression technique in experimental rabbit animal model for cervical spondylotic myelopathy. Ann Med Surg 2021;69:102603.
[12] Ibrahim S, Mousa A, Riawan W. Expression of AIF and caspase-3 in New Zealand rabbit with cervical spondylosis myelopathy model. Ann Med Surg 2021;69:102604.
[13] Impellizzeri D, Esposito E, Mazzon E, *et al*. The effects of a polyphenol present in olive oil, oleuropein aglycone, in an experimental model of spinal cord injury in mice. Biochem Pharmacol 2012;83:1413–26.
[14] Ishikawa T, Suzuki H, Ishikawa K, *et al*. Spinal cord ischemia/injury. Curr Pharm Des 2014;20:5738–43.
[15] Kamil K, Kumar J, Yazid MD, *et al*. Olive and its phenolic compound as the promising neuroprotective agent. J Sains Malaysiana 2018;47:2811–20.
[16] Kanchiku T, Taguchi T, Kaneko K, *et al*. A new rabbit model for the study on cervical compressive myelopathy. J Orthop Res 2001;19:605–13.
[17] Khalatbary AR, Ahmadvand H. Effect ofoleuropein on tissue myeloperoxidase activity in experimental spinal cord trauma. Iran Biomed J 2011;15:164–7.
[18] Khalatbary AR, Zarrinjoei GHR. Anti-inflammatory effect of oleuropein in experimental rat spinal cord trauma. Iran Red Crescent Med J 2021;14:229–34.
[19] Kummer KK, Zeidler M, Kalpachidou T, *et al*. Role of IL-6 in the regulation of neuronal development, survival and function. Cytokine 2021;144:155582.
[20] Laurence DR, Bacharach AL. Evaluation of Drug Activities: Pharmacometrics. Academic Press; 1964.
[21] Mohagheghi F, Bigdeli MR, Rasoulian B, *et al*. The neuroprotective effect of olive leaf extract is related to improved blood–brain barrier permeability and brain edema in rat with experimental focal cerebral ischemia. Phytomedicine 2011;18:170–5.
[22] Omar SH. Oleuropein in olive and its pharmacological effects. Sci Pharm 2010;78:133–54.
[23] Pang KL, Chin KY. The biological activities of oleocanthal from a molecular perspective. Nutrients 2018;10:570.

[24] Pase CS, Teixeira AM, Roversi K, *et al*. Olive oil-enriched diet reduces brain oxidative damages and ameliorates neurotrophic factor gene expression in different life stages of rats. J Nutr Biochem 2015;26:1200–7.
[25] Sarbishegi M, Gorgich EAC, Khajavi O, *et al*. The neuroprotective effects of hydro-alcoholic extract of olive (*Olea europaea L.*) leaf on rotenone-induced Parkinson's disease in rat. Metab Brain Dis 2018;33:79–88.
26] Tu J, Castillo JV, Das A, *et al*. Degenerative cervical myelopathy: insights into its pathobiology and molecular mechanisms. J Clin Med 2021;10:1214.
[27] Villegas AS, Galbete C, González JMM, *et al*. The effect of the Mediterranean diet on plasma brain-derived neurotrophic factor (BDNF) levels: the predimed-navarra randomized trial. Nutr Neurosci 2011;14: 195–201.
[28] Visocchi M, Di Rocco F, Meglio M. Subacute clinical onset of postraumatic myelopathy. Acta Neurochir 2003;145:799–804.
[29] Vogel P, Machado IK, Garavaglia J, *et al*. Polyphenols benefits of olive leaf (*Olea europaea L*) to human health. Nutr Hosp 2015;31: 1427–33.
[30] Ibrahim S, Nasution IFA, Danil M, *et al*. Potential benefit of olive leaf extract in cervical spondylotic myelopathy model. Ann Med Surg 2022;73:103040.