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Effect of Propolis on Neurological Recovery After Experimental Spinal Cord Injury

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ABSTRACT

AIM: To examine the effect of propolis on the healing process in terms of both electrophysiological and ultrastructural parameters in a rat model of experimental spinal cord injury.

MATERIAL and METHODS: Thirty rats were divided into control, spinal cord trauma, and treated trauma groups with 10 rats per group. The rats were sacrificed after 10 days. Before sacrifice, all rats were neurologically assessed by electrophysiological monitoring, and immediately after sacrifice, the spinal cord was examined ultrastructurally by transmission electron microscopy (TEM).

RESULTS: According to the electrophysiological examination, the treatment group was statistically significantly different from the trauma group. However, no statistically significant difference was found between the control and treatment groups. In terms of the TEM examination, the treatment group was significantly different from the trauma group.

CONCLUSION: In this study, propolis was administered just before the induction of trauma, and the findings suggest that the use of propolis has a positive effect on the healing process. This implies that in order to prevent postoperative deficits, this treatment may be preferably applied before spinal cord surgery for trauma.

KEYWORDS: Spinal cord injury, Propolis, Electron microscopy, Ultrastructure

INTRODUCTION

Acute spinal cord injuries are one of the leading causes of permanent disability worldwide (3,20). According to numerous epidemiological studies on its global incidence and prevalence, the incidence of acute spinal cord injury ranges from 10.4 to 83 per million inhabitants (12). In Turkey, the estimated annual incidence was found to be 12.7 per one million in the population with a male to female ratio of 2.5:1. The average age at injury is 35.5+15.1 years (35.4+14.8 for males and 35.9+16.0 for females (8,14). Motor vehicle accidents (50%), falls, occupational accidents (30%), exposure to violence (11%), and sport-related accidents are the most well-known causes of spinal cord injuries (9). Spinal cord injuries may cause complete or incomplete motor and sensory deficits at different levels depending on the level of the lesion. The pathophysiology of spinal cord injuries is complex and involves two phases that comprise primary and secondary injuries (5). Primary mechanical damage results in damage to axonal structures, blood vessels, and cell membranes, which subsequently leads to secondary cell damage with vascular deterioration, edema, ischemia, electrolyte imbalance, release of free radicals, inflammation, and late apoptotic cell death. If during the primary phase of damage, preserved or partially affected cells are protected from mechanisms of secondary damage, the treatment outcomes of the existing pathology will likely be better. Therefore, the primary purpose of many experimental treatment methods is to partially or completely prevent secondary damage (22). For further details on delayed tissue damage, many pathophysiological mechanisms have

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been previously described (4). Moreover, intensive studies on the prevention of trauma-induced apoptosis and the use of antioxidant and neuroprotective agents are ongoing, but additional studies are needed (2).

In our study, propolis was used to prevent trauma-induced apoptosis in a rat model of spinal cord injury. Propolis is a natural remedy that has been widely used since ancient times. Honeybees collect this substance from plant sources, and its biological and pharmacological activities have been studied for many years (16). Caffeic acid phenethyl ester (CAPE) is the most active component of propolis, and its neuroprotective, antioxidant, and antiapoptotic effects have also been shown in several studies (10,11). CAPE is one of the most powerful known lipophilic antioxidants, and as a redox-sensitive and stress-inducible protein, CAPE leads to the release of heme oxygenase-1 (HO-1) (10,24). It has been shown that as the secretion of the HO-1 enzyme increases, organ dysfunction improves due to the prevention of metabolic disorders (17). Currently, no fully effective treatment for secondary injury after spinal cord trauma has been established. The aim of this study was to use electrophysiology and electron microscopy to evaluate the effect of propolis and its antioxidant activity on spinal cord in rats after experimental trauma.

MATERIAL and METHODS

Experimental Animals

Thirty male Wistar Albino rats supplied by the laboratory with normal motor activity weighing 230–260 grams were used in this study. The animals were monitored under standard laboratory conditions and had ad libitum access to food and water; rats were also maintained on a 12-hour light-dark cycle for 10 days before the experimental study was initiated at which point they were treated in accordance with the guidelines of the Local Ethics Committee.

All experimental procedures in this study were approved by the T.C. Ministry of Health University of Health Sciences Ankara Training and Research Hospital Local Ethics Committee for Animal Experiments with the reference number 0051/567. All experiments were performed in the Experimental Animals Laboratory of Ankara Training and Research Hospital.

The rats were randomly divided into 3 groups, as follows: the control group (Group 1, n=10), the trauma group (Group 2, n=10), and the trauma plus treatment group (Group 3, n=10). All rats were sacrificed after 10 days. Before sacrifice, rats were evaluated both clinically and electrophysiologically, and all evaluations were found to be normal. After sacrifice, spinal cord tissue was prepared for examination by electron microscopy. The postoperative clinical tests were consistent with trauma and the neuromonitoring results.

Surgical Procedure

Each rat was anesthetized via intramuscular injection of a combination of ketamine hydrochloride at a dose of 35 mg/kg (Ketalar 5% solution, Eczacibasi, Turkey) and xylazine hydrochloride at a dose of 1.5 mg/kg (Rompun 2% solution, Bayer, Germany). The rats underwent neuromonitoring before, during, and after the procedure, and motor evoked potentials (MEPs) were recorded for each rat.

Then, a midline incision at the thoracic 5, lumbar 4 position was made with the animal in a prone position, and total laminectomy from thoracic 7 to lumbar 2 was performed. The spinal cord of rats in the trauma (Group 2) and trauma plus treatment group (Group 3) was then subjected to trauma according to Allen's weight drop method (Allen, 1914). A sterile lead ball weighing 8 mg was dropped through a 9-cm cylindrical hollow glass tube to induce the standard 72 dynn trauma in each experimental animal.

Preparation of Tissue Samples

After 10 days, the rats were sacrificed under anesthesia, and tissues were prepared for electron microscopy examination.

Propolis Preparation

The water-soluble propolis extract was purchased from Aksuvital Natural Products Food Industry Trade Inc. (Istanbul, Turkey). Based on a study by Koc et al. regarding the protective effects of propolis in rat ovary against ischemia-reperfusion injury, the propolis extract was dissolved in 100 ml distilled water and used at a concentration of 200 mg/kg (15). A 1-cc solution was administered by gavage only to rats in Group 3 one hour before trauma induction. The dose was selected based on the literature and on our preliminary studies.

Transmission electron microscopy tissue preparation technique

The tissue samples were placed into 2.5% glutaraldehyde for 24 hours for primary fixation. Then, the samples were washed with Sorenson's Phosphate Buffer (pH: 7.4) and post-fixed in 1% osmium tetroxide for 1 hour. After post-fixation, the samples were washed in the same buffer and dehydrated in increasing concentrations of alcohol. After dehydration, the tissues were washed with propylene oxide and embedded in epoxy resin embedding media. Semi-thin and ultra-thin sections of the resulting tissue blocks were cut using an ultramicrotome (LKB Nova, Sweden). These semi-thin sections, which were 2 micrometers in thickness, were stained with methylene blue and examined by light microscopy (Nikon, Japan). Following this procedure, the tissue blocks were trimmed, and ultra-thin sections, which were approximately 60 nanometers in thickness, were obtained using the same ultramicrotome. These ultra-thin sections were stained with uranyl acetate and lead citrate and examined with a Jeol JEM 1200 EX (Japan) transmission electron microscope. Electron micrographs of the specimens were obtained using the same microscope.

RESULTS

The data in this study were evaluated according to two parameters: electrophysiology and electron microscopy. Electrophysiological assessments of the rats revealed no statistical preoperative difference among the groups; for the later evaluation, the differences between Group 1 and Group 2 were statistically significant, whereas Group 3 was not significantly different from the other groups (Table I, Figure 1).

Table I: Mean Amplitude and Standard Deviation of Groups

GROUPS/ MEP (mean mV and ± Std Deviation)	Preoperative amplitude	Amplitude after laminectomy	Amplitude after trauma	Late period amplitude
Group I (Control)	371.280 ± 134.59	268.28 ± 121.53	(-)	325.10 ± 134.75
Group II (Trauma)	381.80 ± 133.41	304.59 ± 40.24	34.02 ± 9.03	82.03 ± 27.94
Group III (Trauma + Treatment)	447.31 ± 63.66	338.59 ± 40.24	46.54 ± 11.34	306.74 ± 208.14

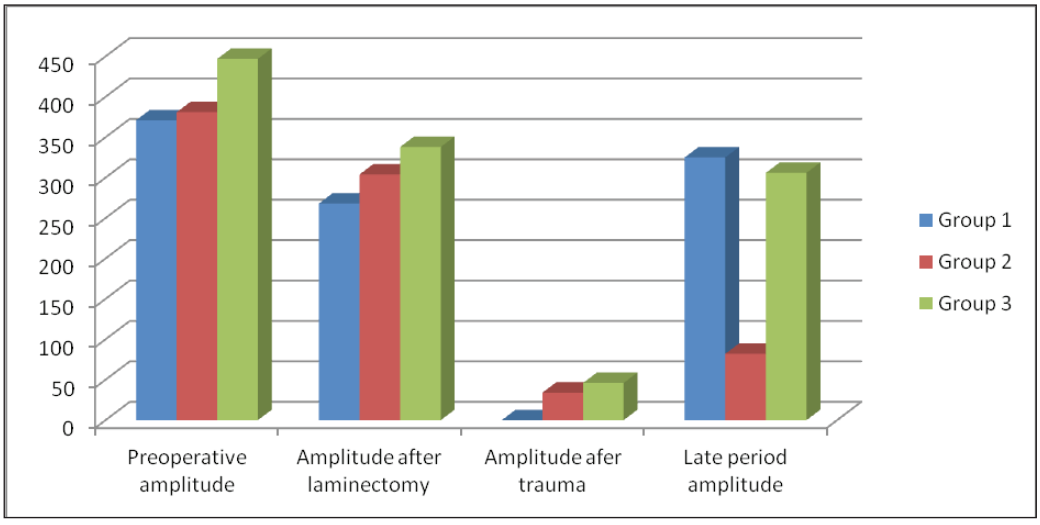

Figure 1: Average amplitude values of groups.

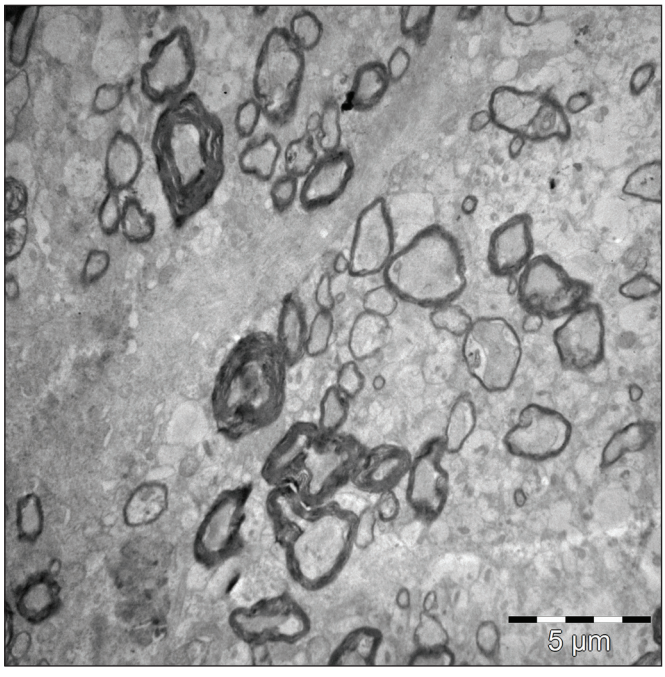

Figure 2: Laminectomy (x6000).

When the samples from Group 1 were examined by TEM, no ultrastructural pathology was detected (Figure 2). However, severe intercellular edema was detected between the neurons and grey matter, and large vacuoles were observed inside the cytoplasm of neurons in Group 2 samples. Additionally, the mitochondria appeared swollen (Figure 3A, B). When samples from Group 3 were examined by TEM, the degree of intercellular edema was found to be smaller compared with that in Group 2. Moreover, vacuoles were also present inside the neuronal cytoplasm, and a portion of the mitochondria was swollen. However, these vacuoles were smaller in size compared with those in Group 2 (Figure 4A, B). Perineuronal edema was not present in the propolis-treated group. The ultrastructure of the neurons and grey matter in Group 3 samples exhibited a very prominent and marked difference from that of Group 2. Non-myelinated axons in samples from all three groups exhibited normal ultrastructure.

DISCUSSION

Spinal cord injury is recognized as one of the primary causes of permanent disability, as it results in significant physical damage as well as psychosocial and economic repercussions**.** A universally accepted treatment protocol has not yet been established and is thus an important issue (13). Efficient pharmacological treatment methods that are in development clearly show the need to understand the pathophysiology of post-traumatic processes. Dramatic changes in the pathological appearance of spinal cord lesions within the first few days after injury constitute the most important clinical and experimental observation points (21). First, in 1911, Allen reported a contusion-type spinal cord injury in dogs followed by myelotomy (1). The removal of post-traumatic hematomyelia improved

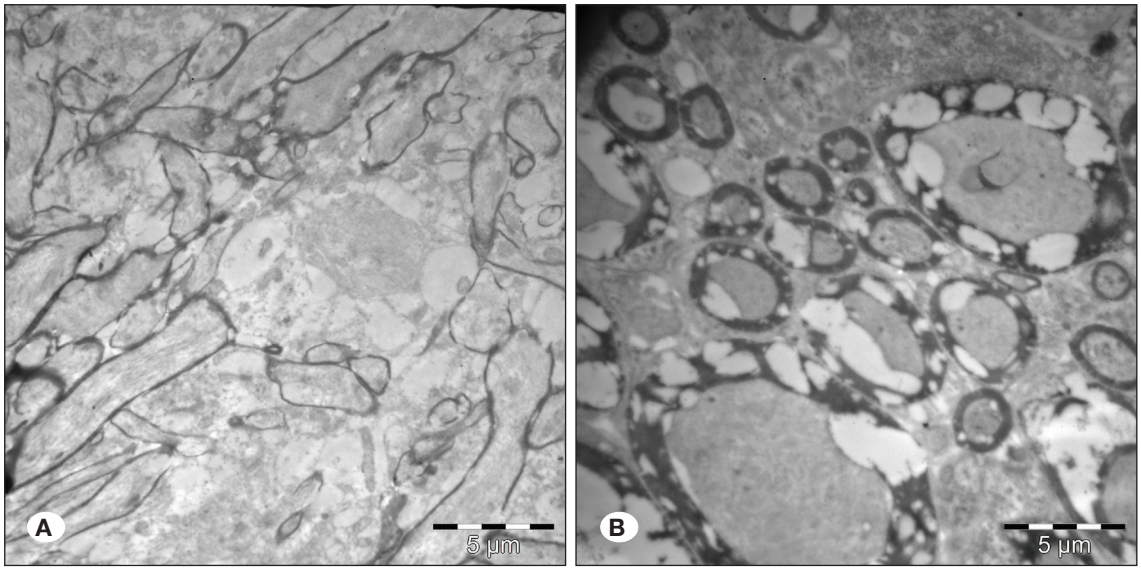

Figure 3:
A) Trauma (x6000).
B) Trauma (x6000).

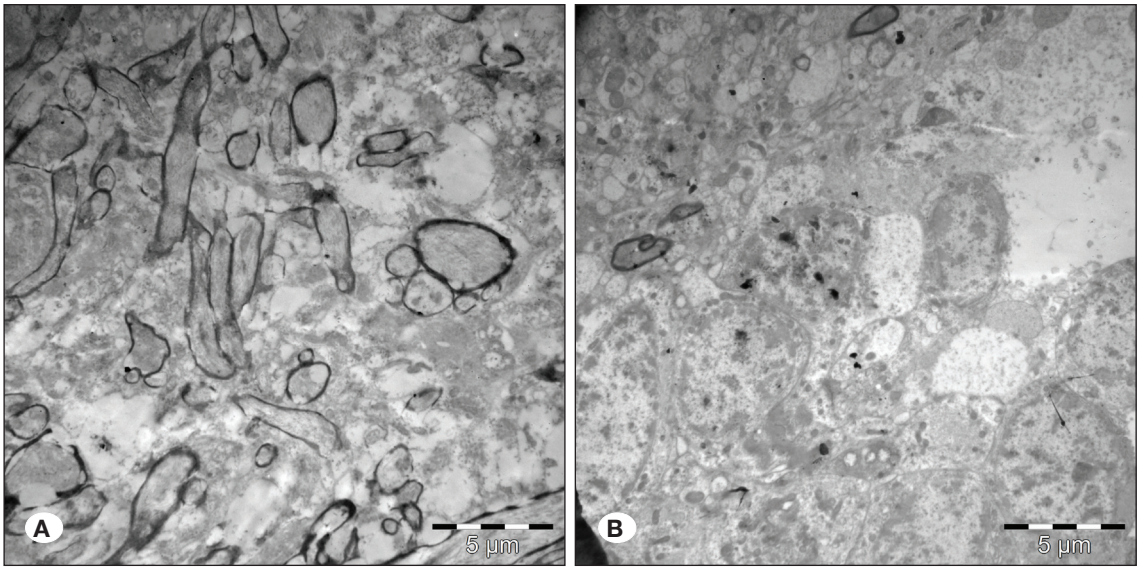

Figure 4:
A) Propolis (x6000).
B) Propolis (x6000).

the neurological function of the animals, and through his research, he pioneered the concept of secondary damage by ensuring that his experimental studies on this subject were based on specific criteria. Since then, various experimental models have been developed to investigate the pathophysiology of spinal cord injury and to evaluate the effects of neuroprotective agents (25). Although the ability of many of these models to fully replicate an injury in an experimental setting is doubtful, in many animal models, damage is induced by dropping a free weight from the posterior over an open laminectomy, which we applied in our study. In the experimental trauma studies published to date, different pharmaceuticals have been administered after trauma and assessed as potential treatments. Historically, data have been measured by functional, anatomical, neurophysiological, radiological, biochemical, histopathological, and electrophysiological means, and theories of new treatment methods have been established. In our study, electrophysiological and histopathological data were obtained using intraoperative neuromonitoring in accordance with the literature, and the usefulness of the treatment was evaluated (6). In this study, we treated a subset of rats with propolis before trauma induction. We know that spinal cord trauma is unpredictable and emergent, and unfortunately, we also know of the probability of postoperative deficits after spinal cord surgeries (7,18,19). Considering the possibility of postoperative deficits in elective surgeries such as those for scoliosis, spinal canal stenosis surgeries, spinal cord tumors, or tumors that compress the spinal cord, we believe that propolis may be beneficial if administered before a surgical procedure. In our study, it is predicted that the morbidities that may develop after such spinal cord surgeries can be reduced by the advance administration of propolis.

Various electrophysiological parameters can be recorded such as MEP, SSEP, dSSEP, EMG, and SSR (23). In our study, we assessed the electrophysiological data with an intraoperative neuromonitoring device, which is increasingly used during routine brain and nerve surgeries.

With this application, which also provides monitoring during surgical procedures, standardization between the groups can be monitored during the laminectomy and trauma application stages**.** In the present study, no statistically significant difference was found among the three groups in the preoperative amplitudes of the stimulated muscle groups, whereas a statistically significant decrease in the amplitudes in the muscle groups measured after laminectomy was found among all groups.

When the amplitude values measured after laminectomy were evaluated and compared among the groups, no statistically significant difference was found between any of the groups in terms of amplitude both before and after laminectomy.

This shows that during the laminectomy, while this process generated unintentional damage and caused significant amplitude reduction in all groups, the damage did not result in a statistically significant difference among the groups. No statistically significant difference in the amplitudes after trauma or in the decreases in amplitude after trauma was observed among the two trauma groups.

These findings led us to conclude that more rigorous surgery and the use of more appropriate surgical instruments are crucial and revealed the importance of intraoperative monitoring to confirm that standardization has been achieved in similar experimental models. The late amplitudes of the rats in Group 3, which received propolis, were significantly higher than those of Group 2, in which trauma was induced without propolis administration. However, no statistically significant difference was observed between the late amplitudes and the post-traumatic amplitudes of the control group, which had only laminectomy and whose amplitudes had decreased significantly.

CONCLUSION

In this study, propolis was administered just before trauma induction, and the findings suggest that the use of propolis has a positive effect on the healing process. This implies that in order to prevent postoperative deficits, the use of propolis before surgery may be preferable to reduce the risk of injury to the spinal cord such as during surgeries to remove spinal cord tumors.

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