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ORIGINAL ARTICLE

Neurotoxicity of various root canal sealers on rat sciatic nerve: an electrophysiologic and histopathologic study

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Abstract
Objectives The aim of this study was to compare the neurotoxicity of various root canal sealers on rat sciatic nerve by electrophysiologic and histopathologic analyses.
Materials and methods A total of 40 male rats were randomly divided into five groups: Control, AH Plus, GuttaFlow, Sealapex and Smartpastebio. Sciatic nerves of the rats were uncovered using the surgical procedures, and the prepared sealers were then applied on nerves with a polyethylene tube vehicle for 15 days. Nerve potentials were recorded at initial exposure, 5, 30 and 120 min (early phase), and 15 days (late phase) by an electrophysiologic analysis system for all groups. The obtained measurements were then used to calculate the nerve conduction velocities (NCV). Subsequently, all rats were sacrificed, and their sciatic nerves were removed for histopathologic analysis. Statistical analysis was performed using the Kruskal-Wallis and Mann-Whitney *U* tests for intergroup variables and the Friedman and Wilcoxon test for intragroup variables. Statistical significance was set at $P<0.05$.
Results There was no significant difference between early and late phase results in the control group. This group showed little or no lasting damage to nerve tissue. All sealers decreased the NCV in the early phase time periods, but this decrease was only statistically significant in the AH Plus group at 120-min time period ($P<0.0125$). During the late phase, the AH Plus and GuttaFlow groups almost reached initial NCV values, and it was lower than the initial values in the Sealapex and Smartpastebio groups. However, this decrease was not statistically significant. When intergroup comparisons were performed, statistically significant differences occurred at 30 min in the Sealapex group and 120 min in the AH Plus group compared with the control group ($P<0.0125$). All sealers induced neurotoxicity as a result of degenerative and inflammatory responses of nerve tissue in histologic analysis. Histologic analysis revealed Sealapex and GuttaFlow to be the most and least neurotoxic, respectively.
Conclusions All tested root canal sealers exhibited a variable degree of neurotoxicity depending on their chemical compositions.
Clinical relevance Apical extrusion of endodontic filling materials may cause undesired consequences, such as inflammation and severe neurotoxic damage; therefore, extrusion factor plays an important role during the root canal treatment.

Keywords Neurotoxicity · Root canal sealer · AH Plus · GuttaFlow · Sealapex · Smartpastebio · Rat sciatic nerve · Animal study

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Introduction

Endodontic treatment depends on completely cleaning and shaping, and then filling the root canals. During this procedures, overinstrumentation, irrigants, intracanal dressings, infected debris, bacteria, dentin chips, or filling materials including sealers may be extruded into the periradicular or neigboring tissues (e.g., maxillary sinus or mandibular canal) [1–3]. Therefore, extrusion cases, whether intentional or unintentional, can cause several problems ranging from inflammation to severe neurotoxic damage [4]. Neurotoxic damage may be by mechanical, thermal, or chemical mechanisms. As a result of this damage, clinical sensory disorders such as pain, hyper/hypoaesthesia, anaesthesia, dysaesthesia and paraesthesia can occur [5]. It has been reported [6] that neurotoxic endodontic materials cause changes in nerve membrane potential and transient or permanent block by inhibiting action potential conduction, which is the basis of these sensory disorders. The effect of chemical damage on the nerve is related to level of content toxicity and removal rate of used fillings from the region. Pogrel [4] mentioned a direct relationship between the amount of time that the material remains on the nerve and prognosis of paraesthesia. For these reasons, early diagnosis and prompt management to reduce the risk of permanent damage is critical.

The most common canal filling extrusion cases discussed in published literature are related to mandibular teeth [7, 8]. Inadequate endodontic treatment (overextension and/or overfilling) of these teeth can damage the inferior alveolar nerve (IAN) bundle due to close proximity to the related roots [7–9]. Apart from its distance to the root apex, the risk of this type damage is dependent on several other factors, such as clinical tooth angulation, position of the anatomical foramen, the presence of accessory foramina, the presence/absence of cementum around the periapex, low quality and density of the trabecular bone and the degree of cortication of the IAN [9, 10]. There have been reports of IAN damage in up to 1 % of cases when performing defective root canal treatment of mandibular premolars [10]. The risk of this type of damage is also reported to be greater with mandibular second molars compared with mandibular first molars [11]. Chong et al. [9] showed that there was an intimate relationship between the roots of the mandibular second molars and the IAN in over 50 % of the cases. Therefore, necessary precautions should be taken during the treatment.

Root canal sealers (RCSs) are an essential part of filling; not only do they provide for an adequate seal between the core material and the dentin, but they are also effective antimicrobials inside the root canals [12]. In addition to defined requirements of mechanical and physical properties, the biocompatibility of sealers is important because they come into contact with periradicular tissues [13]. To find an ideal sealer, many materials have been developed based on glass ionomer, zinc oxide eugenol, calcium hydroxide/silicate, epoxy/methacrylate resins and silicone for filling. Most of them have shown inadequate biological activity and have been evoked variable degrees of toxicity depending on their chemical compositions [14–16]. To date, many studies [14–18] assessed the cytotoxicity of RCSs, although only a few authors observed the effects of extruded materials on the neurotoxicity of them [19–24]. This animal study was designed to assess and compare the possible neurotoxicity of four RCSs [AH Plus (an epoxy resin-based sealer, Dentsply De Trey, Konstanz, Germany), GuttaFlow (a silicone-based sealer, Colthane/Whaledent, Langenau, Germany), Sealapex (a calcium hydroxide-based sealer, SybronEndo, MI, USA) and Smartpastebio (a calcium silicate-based sealer, DRFP, Stamford, UK *also known as* iRoot SP sealer, Innovative Bioceramix Inc., Vancouver, Canada)] on rat sciatic nerve using electrophysiologic and histopathologic analyses.

Materials and methods

Tested sealers

Four RCSs (AH Plus, GuttaFlow, Sealapex and Smartpastebio) were tested. Composition and manufacturer of tested sealers are listed in Table 1.

Surgical procedure

The Declaration of Helsinki recommendations and internationally and locally accepted principles were followed for the care and use of the animals in this study. All study protocols were approved by the local ethics committee (KTU 2011/26). Forty adult male Sprague-Dawley rats weighing 300–350 g were used. The animals were housed in groups of 3–4 and were allowed free access to food and water, except for the short time that the rats were removed from their cages for the experiments. All rats were kept in a temperature-controlled (22±1 °C) environment on a 12-h light/dark cycle.

Rats were anaesthetized with an intraperitoneal (i.p.) injection of ketamine (100-mg/kg body weight) (Ketalar; Eczacibasi, Istanbul, Turkey) and Rompun (7-mg/kg body weight) (2 % Xylasin; Bayer, Istanbul, Turkey). Additional doses were given as needed. The lateral aspect of the left thigh, hip and flank was then routinely prepared, including trimming of the hair and antisepsis.

Table 1 Composition and manufacturer of the tested sealers

Material	Composition	Lot number	Manufacturer
AH Plus *(epoxy resin-based)*	Paste A: Bisphenol-A and -F epoxy resins, calcium tungstate, zirconium oxide, silica, iron oxide pigments. Paste B: Amines, calcium tungstate, zirconium oxide, silica, silicone oil.	1106000705	Dentsply De Trey, Konstanz, Germany
GuttaFlow *(polydimethylsiloxane-based)*	Gutta-percha powder, polydimethylsiloxane, silicone oil, paraffin oil, hexachloroplatinic acid, zirconium oxide, nano-silver (preservative).	6308281	Colthane/Whaledent, Langenau, Germany
Sealapex *(calcium hydroxide-based)*	Paste A: Isobutyl salicylate resin, silicon dioxide, bismuth trioxide, titanium dioxide pigment. Paste B: N-ethyl toluene sulfonamide resin, silicon dioxide, zinc oxide, calcium oxide.	1-1267	SybronEndo, MI, USA
Smartpastebio *(calcium silicate-based)*	Zirconium oxide, calcium silicates, calcium phosphate, calcium hydroxide, filler and thickening agents.	10003SP-EU	DRFP, Stamford, UK*also known as* iRoot SP sealer, Innovative Bioceramix Inc., Vancouver, Canada

The sciatic nerve was uncovered through a posterolateral longitudinal straight incision going down from the greater trochanter to the lateral condyle of the femur, followed by blunt dissection between the gluteus superficialis and the biceps femoris. The entire length of the nerve was made visible, and its three main distal branches, the common peroneal, tibial and sural nerves, were carefully identified in the popliteal fossa. It was uncovered approximately 2 cm of the sciatic nerve above its trifurcation without injuring the epineurium (Fig. 2a). Demonstration of experimental setup is shown in Fig. 1. Animals were then randomly assigned to four experimental

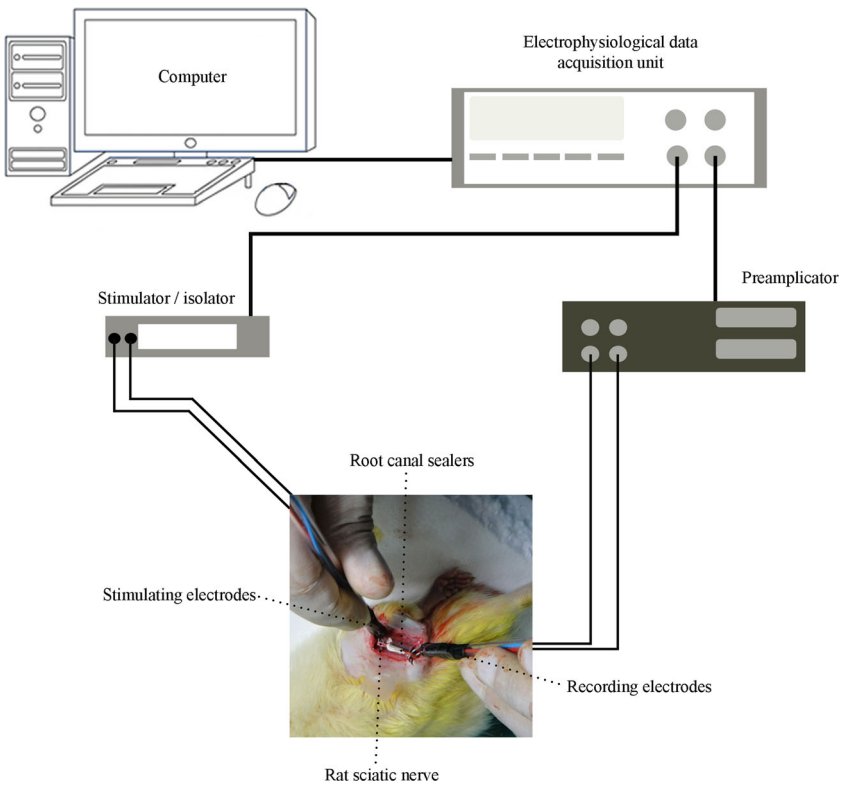


Fig. 1 Demonstration of experimental set-up

groups (n=8) according to sealer type and one control group (n=8).

Preparation of tested sealers

To standardize the contact area between the sealer and sciatic nerve and to reduce the contact of sealer with surrounding tissues to a minimum, a 4-mm diameter 0.5-cm-long sterile and nontoxic polyethylene tube (Butterfly Set; YC Medical, Ankara, Turkey) was used (Fig. 2b). To provide contact between sealer, which was placed into tube, and sciatic nerve, a longitudinal cut was made along the length of the tube with a sterile scalpel.

All sealers were prepared according to the manufacturer's recommendations, and they were placed into the polyethylene tube carefully as to avoid any air formation in the tube. After, the longitudinally cut plastic polyethylene tubes were filled with sealer. The amount of sealer was 0.18 g for each tube. They were then placed on the sciatic nerve, which had been isolated with a thin paraffin strip.

Electrophysiologic analysis

The recordings of nerve potentials were carried out using an electrophysiologic data acquisition system (PowerLab 16/30; AD Instruments, Castle Hill, Australia). Bipolar hook electrodes were used to stimulate the left sciatic nerve and to record the nerve potentials (Figs. 1 and 2c). For the first stimulating point, a hook electrode was placed proximally to the sciatic notch. The recording electrode was placed distally on the sciatic nerve. After creating stimulus and recording at the first point, the distance between stimulus electrode and recording electrode was measured twice with a caliper. Later, the stimulus electrode was moved approximately 1 cm distal from the first point, creating a second stimulus point a supramaxillar impulse was sent to the sciatic nerve for a 2-ms duration from a stimulus on the PowerLab 16/30 data recording system. The distance between stimulus electrode and recording electrode was measured twice with a caliper (Fig. 2d). All responses were amplified with an amplifier (ML136 Animal BioAmp; AD Instruments) and stored on a computer (Fig. 1). A software (Scope v3.9.1; AD Instruments) was used for data capture and analysis. The latencies were measured from the stimulus artifact to the onset of first wave deflection. Nerve conduction velocities (NCVs) were calculated by dividing the distance between stimulating sites by the average latency difference between the onsets of the compound action potential (CAP). The amplitude of the CAP was measured from peak to peak. To determine the latency, electrical stimulation was repeated 10 times and averaged per rat. Electrophysiologic recording procedure was performed in the same way for all groups. The first measurements (base measurement) made on the sciatic nerve were taken just before the sealer filled plastic polyethylene tube was placed on the sciatic nerve, then 5 min after the placement and again at 30 and 120 min. All these masurements are named early phase measurements. Control group measurements were made without any applied procedure on the nerve. During all measurement periods, to protect the tissue, the related area was

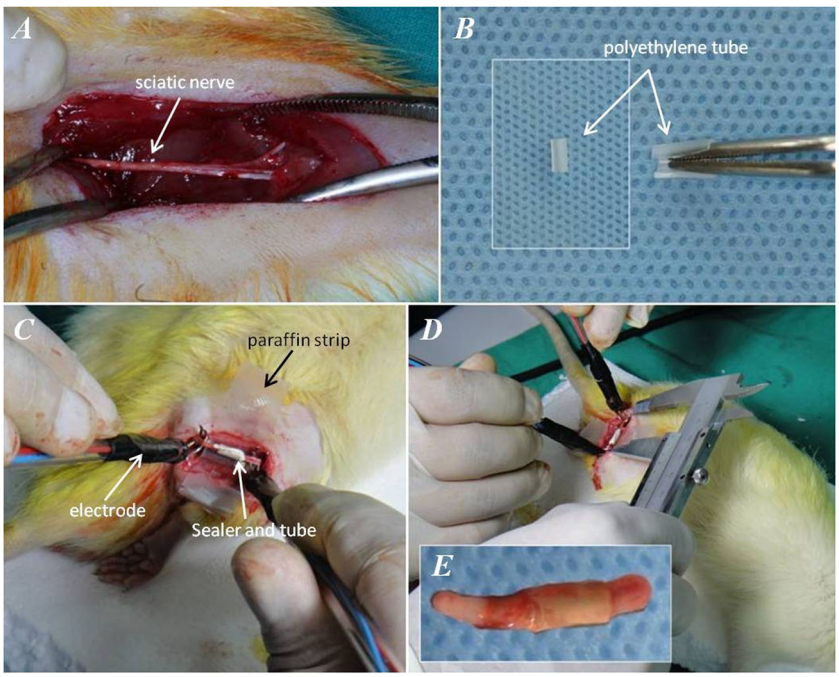


Fig. 2 **a** Uncovering of the rat sciatic nerve. **b** Sterile and nontoxic polyethylene tube for the transportation and placement of the sealers. **c** Electrophysiologic recordings with electrodes. **d** Measurement of the distance between stimulus and recording electrodes with a caliper. **e** An extracted sciatic nerve tissue for histopathologic analysis

irrigated with sterile saline solution every 10 min. After 120 min, measurements were completed, and the placed tube filled with sealer was left in place. Wound areas were closed with silk sutures, and the rats were put on a 15-day recovery period.

At the end of the 15 days (late phase measurement), rats were once again anaesthetized, the same surgical procedures were performed and the sciatic nerve was uncovered again. For the late phase measurements, the previously described electrophysiologic recordings were repeated in all groups. Because sciatic nerve was detached during the uncovered procedure in two rats in Sealapex group, one rat in GuttaFlow group and one rat in Smartpastebio group the late period measurements could not be made. For this reason, these rats were excluded from the study.

Histopathologic analysis

At the end of the 15 days, the late phase electrophysiologic recordings were obtained, and afterward, all rats were sacrificed with servical dislocation. For histopathologic and histomorphologic analysis, the sciatic nerve was extracted as to include the not contacted with sealer from the proximal and distal region where the implants were placed and then tube used for fix the sealer was removed from the nerve (Fig. 2e). Sciatic nerve tissue samples were immersed to 10 % buffered formalin for 24 h at room temperature. The fixed tissues were washed in running tap water for 4–8 h, which were kept for tissue follow-up after soaking in increasing alcohol series (% 50–100) and xylene. Tissues were then embedded in paraffin and blocked. The sections were cut at a thickness of 4–5 μm with a microtome (RM2255; Leica, Solms, Germany) and prepared on glass slides for analysis. Sections were stained with haematoxylin/eosin and luxol fast blue stains and then examined under a light microscope (BX51; Olympus, Tokyo, Japan) with digital camera (DP72; Olympus) and photographed at×20 and× 40 magnifications.

Histopathologic evaluation and scoring

Evaluations of all groups were performed in three stages. In first stage, nerve tissue was evaluated with regard to myelin, axon and epineurium degeneration. In second stage, nerve tissue was evaluated with regard to fibrosis and thickening degree of epineurium and perineurium. In third stage, tissue was evaluated with regard to lymphocyte infiltration, fibrocyte density, vacuolization and edema. Scoring was blindly graded in all three stages by two skilled examiners with a 6-point scoring system, grading them as no (0 point), mild (1 point), moderate (2 point), significant (3 point), severe (4 point) and very severe (5 point). Histopathologic parameters were evaluated subjectively by these calibrated examiners.

Statistical analysis

All statistical procedures were performed using a statistical software program (SPSS v15.0; SPSS Inc., Chicago, IL, USA). Statistical analysis was carried out using Kruskal-Wallis, Mann-Whitney *U*, Friedman and Wilcoxon tests. Statistical significance was set at $P<0.05$.

Results

Electrophysiologic findings

There was no significant difference between early and late phase results in the control group. This group showed little or no lasting damage to nerve tissue. According to the early phase results, all sealers showed a decrease in NCV values. However, this reduction was only statistically significant in the AH Plus group during intragroup evaluations when initial values were compared with 120-min values ($P<0.0125$) (Table 2). During the late phase, the AH Plus and GuttaFlow groups almost reached initial NCV values, and it was lower than the initial values in the Sealapex and Smartpastebio groups. However, this decrease was not statistically significant. When intergroup comparisons were performed, statistically significant differences occurred at 30 min in the Sealapex group and 120 min in the AH Plus group compared with the control group ($P<0.0125$).

Histopathologic findings

Histologic changes were recorded from harvested sciatic nerve tissue samples as shown in Fig. 3 and Table 3. During evaluation of nerve sections obtained from the control group under light microscope, no histologic changes occurred; they were classified as tissue and cells with normal morphologic characteristics. For the AH Plus group, varying degrees of axon and myelin degeneration was observed in all sections while mild or moderate epinerium degeneration and inflammation was observed in some sections. Moderate degrees of lymphocyte infiltration, edema, fibrosis, granulation formation, vasodilatation and dense eosinophil infiltration were observed in most of the harvested nerves. It was discovered that the most severe epineurium degeneration (62.5 %), fibrosis (25 %) and edema (50 %) occurred in the AH Plus group. Mild or moderate axon and myelin degeneration was observed in the nerves applied with GuttaFlow. In addition, while epineurium degeneration was not observed, fibrosis and granulation formation at

Table 2 Comparison of nerve conduction velocity in the groups and determination of significance

	n	Nerve conduction velocities (m/s)					Intragroup *P* values
		Initial	After 5 min	After 30 min	After 120 min	After 15 days	
Control	8	45.76±12.32	46.41±09.57	54.22±14.19	48.43±16.70	49.94±13.73	.699
AH Plus	8	45.51±15.84	39.61±14.42	34.61±15.22	24.43±07.48**	43.65±09.05	.002
GuttaFlow	7	45.77±11.18	35.62±17.22	39.77±08.87	34.86±08.30	45.20±13.50	.348
Sealapex	6	47.90±23.19	34.82±14.11	31.13±13.26*	31.24±16.41	31.90±09.84	.388
Smartpastebio	7	46.05±16.99	45.96±10.92	51.74±16.91	32.63±17.50	37.70±14.04	.093
Intergroup *P* values		.999	.246	.022	.036	0.152	

*P=0.022, shows significance between Sealapex and control groups for "After 30 min" session

**P=0.036, shows significance between AH Plus and control groups for "After 120 min" session

**P=0.002, shows significance between "After 120 min" and "Initial" sessions for AH Plus group

inflammation sites and marked edema were detected. The GuttaFlow group exhibited the most severe epineurium thickening (28.6 %) and lymphocyte infiltration (42.8 %). Epineurium and endoneurium degeneration, edema, perinuclear lymphocyte infiltration, fibrosis, thickening of supply arteries to the nerve and vacuolizations were observed in the Sealapex group. While severe myelin and axon degeneration was observed near the contact region, mild degenerations were observed in regions further from sealer contact. It is obvious that the most severe myelin and axon degeneration (50 %) and the most severe fibrocyte density (50 %) were observed in the Sealapex group. Prominent thickening of the epineurium and perineurium, granulation formation, fibrosis, dense myelin and axon degeneration and edema were observed in the Smartpastebio

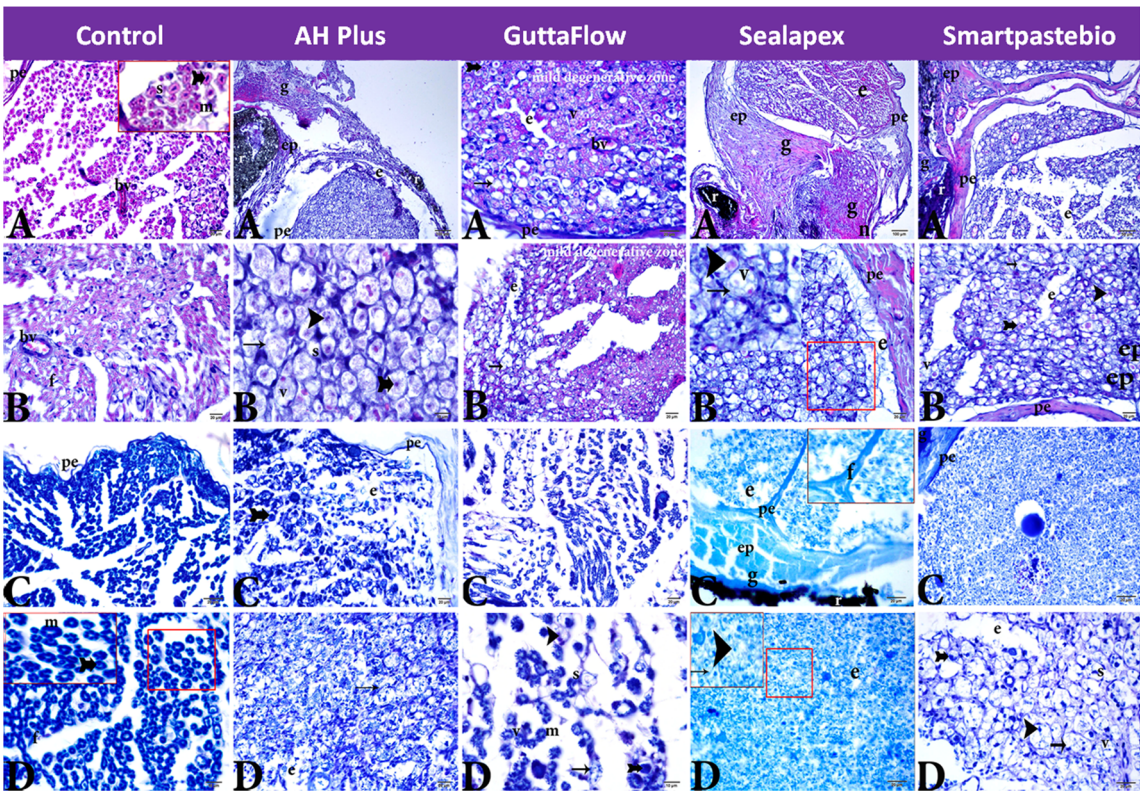


Fig. 3 Histopathologic examination of control, AH Plus, GuttaFlow, Sealapex and Smartpastebio groups in light microscope. *pe* perineurium, *s* Schwann cells, *m* myelin, *thick arrow* axon, *e* oedema, *bv* blood vessel, *f* fibrocyte, *v* vacuolization, *arrowhead* axonal degeneration, *thin arrow* myelin degeneration, *g* granulation tissue, *r* residual sealer substances. Magnifications, AH Plus-A and Sealapex-A (×10); Smartpastebio-A (×20); control-C (×60); AH Plus-B, control-D and GuttaFlow-D (×100); others (×40). Images **a** and **b** H&E and **c** and **d** Luxol fast blue stains. The difference between these stains; Luxol fast blueis indicates the better myelin degeneration

Table 3 Histologic scoring results of sciatic nerves after 15 days of exposures

	Control						AH Plus						GuttaFlow						Sealapex						Smartpastebio						*P*
	G0	G1	G2	G3	G4	G5	G0	G1	G2	G3	G4	G5	G0	G1	G2	G3	G4	G5	G0	G1	G2	G3	G4	G5	G0	G1	G2	G3	G4	G5	
Myelin degeneration	5	3	-	-	-	-	-	-	3	3	2	-	-	2	3	1	1	-	-	-	-	-	3	3	-	-	1	2	4	-	.000**
Axon degeneration	6	2	-	-	-	-	-	1	5	2	-	-	-	4	2	1	-	-	-	-	-	3	3	-	-	3	2	2	-	-	.000**
Epineurium degeneration	5	3	-	-	-	-	-	1	2	5	-	-	5	2	-	-	-	-	-	-	2	2	2	-	2	2	3	-	-	-	.000**
Fibrosis	6	2	-	-	-	-	-	4	2	2	-	-	1	4	1	1	-	-	-	-	-	2	3	1	-	-	3	3	1	-	.000**
Epineurium thickening	7	1	-	-	-	-	2	2	3	1	-	-	-	3	2	2	-	-	-	5	1	-	-	-	-	2	4	1	-	-	.000**
Perineurium thickening	6	2	-	-	-	-	1	3	3	1	-	-	1	2	3	1	-	-	1	3	2	-	-	-	-	3	4	-	-	-	.000**
Lymphocyte infiltration	5	3	-	-	-	-	-	1	4	3	-	-	-	1	1	2	3	-	-	1	-	3	2	-	-	-	3	3	1	-	.001*
Fibrocyte density	6	2	-	-	-	-	-	1	3	3	1	-	-	1	2	3	1	-	-	-	1	2	3	-	-	2	3	2	-	-	.000**
Vacuolization	5	3	-	-	-	-	-	3	4	1	-	-	1	2	2	2	-	-	-	2	3	1	-	-	-	2	3	2	-	-	.007
Edema	5	3	-	-	-	-	-	1	3	4	-	-	-	1	4	2	-	-	-	2	2	2	-	-	-	2	3	2	-	-	.002*

P significance level, *G* grade, (G0) no, (G1) mild, (G2) moderate, (G3) significant, (G4) severe, (G5) more severe

*$P<0.005$

**$P<0.001$

group. The most severe perineurium thickening was also observed in Smartpastebio group (57.1 %).

Discussion

Extrusion of sealers has been shown to have cyto-, geno- and neurotoxic effects on the periradicular or close anatomical tissues [5]. Neurotoxicity of sealers has been previously researched [19–24]. For the first time, Brodin et al. [19] evaluated the effect of certain sealers on rat phrenic nerve action potential conduction. After, Boisen and Brodin [20] showed that calcium hydroxide-based sealers induced a fast and complete inhibition of CAP on rat phrenic nerve. Using the same technique, the neurotoxicity of RCSs-Endomethasone, N2 Universal, Traitement SPAD, Sealapex and CRCS was researched on isolated rat sciatic nerves after local application [22]. Authors founded that all tested sealers reversibly inhibit the CAP amplitudes. Asgari et al. [23] compared the changes that occurred with use of AH 26 and Roth 801 sealers on the action potential of snail F1 neurons and evaluated the behaviour and electrical excitability of nerves after applying the sealer directly or in dissolved form to the solution where the nerve was placed. As a result, it was reported that AH 26 had a 2-way effect; its early phase effect is stimulating; however, later the sealer created significant inhibition in excitability and electrical behavior. A recent study [24] demonstrated that the sealers can directly activate trigeminal nociceptors, leading to a robust release of calcitonin gene-related peptide, and may therefore lead to pain and neurogenic inflammation.

Neurotoxic effects of AH Plus, GuttaFlow, Sealapex and Smartpastebio RCSs were evaluated on rat sciatic nerve tissue in this study. We used rat sciatic nerves, because it was a mixed nerve, similar to the IAN, easy to expose surgically without causing any damage, and had a thick-enough nerve bundle to facilitate electrophysiologic recordings.

When the RCSs contacted with a nerve tissue, it has been reported [25] that the sealers effect nerve transmission in 2 ways. The first way is that ionic imbalance in the extracellular fluid surrounding the axons can lead to temporary conduction block; this state is reversible and recovery is fast and complete. The second way, which is more severe, is where chemical damage leads to axonal degeneration in the distal region of the damaged area and impulse conduction ceases. In this type of injury, recovery is slow, and complete recovery rarely occurs. For this reason, in order to evaluate immediate and long-term effects of sealers on the rat sciatic nerves, a total of five electrophysiologic recordings were taken for each rat, 4 times during the early phase and once during the late phase. Recordings were taken prior to sealer application, 5, 30, and 120 min (early phase) after application, and finally 15 days later (late phase). Furthermore, to determine possible nerve tissue damage from surgical and electrophysiologic recording procedures, identical interval recordings were taken for the control group, which did not use sealers.

In this study, the CAP amplitude values were recorded during electrophysiologic measurements to calculate the NCV. The CAP is a parameter that is influenced by many environmental factors, such as the types of stimulating and recording electrodes, acute or chronic study and nerve and the electrode contact surface. For this reason, we have chosen the NCV as a primary parameter.

According to the early phase electrophysiologic findings of this study, all sealers were found to decrease NCV in rat sciatic nerves. However, although this decrease was not found to be statistically significant in the GuttaFlow, Sealapex and Smartpastebio groups, there was a statistically significant decrease at 120 min in the AH Plus group. While NCV after 15 days reached initial nerve exposure levels in the AH Plus and GuttaFlow groups, it was lower than the initial levels in the Sealapex and Smartpastebio groups. However, this decrease was not significant statistically. There was no significant difference between early and late phase results in the control group. This group showed little or no lasting damage to nerve tissue. Therefore, we concluded that the surgical procedures used to uncover the sciatic nerve and the trauma caused by the electrodes on nerve tissue did not affect NCV. AH Plus caused reversible conduction block as evidenced by the fact that NCV in this group decreased at 120 min and returned to initial exposure values during the late phase. It is known that AH Plus causes minimal formaldehyde release during stiffening [26]. Grossman and Tatoian [27] have reported that using sealers that have paraformaldehyde in them is risky and can impair sensorial conduction when itis exposed to nerve tissue. The reversible damage caused by AH Plus can be linked to the extremely low amounts of formaldehyde that is released only during stiffening. Early phase reductions observed in Sealapex remained unchanged at the end of 15 days; this showed that this sealer had a continued effect on the nerve.

Among these RCSs, an epoxy resin-based sealer AH Plus is thought to be the “gold standard”. However, according to the results of this study, it was found that this sealer created a mild to significant level of irritation on nerve tissue and also led to an inflammatory response. As with all tested sealers, observed degeneration and inflammation were more pronounced in the superficial fascicles, where the sealer made direct contact, than in the central nerve bundle fascicles. In previous studies [18, 28], AH Plus was shown to have proinflammatory activity. Zoufan et al. [29] demonstrated that while AH Plus showed severe cytotoxicity, once mixed, this toxic effect decreased after stiffening. In another study [30], it was demonstrated that severe toxicity continued for the first 3 days but abated after 3 weeks and then became nontoxic. Post-mixing high cytotoxicity has been attributed to the release of formaldehyde or epoxy resin compounds. Contrary

to these studies, there have been other studies [31, 32] that have reported that AH Plus sealers caused mild to no cellular damage.

GuttaFlow is a relatively new polydimethylsiloxane-based sealer used in root canal treatments. In this study, it was observed that while GuttaFlow generally displayed only a mild to average degenerative effect, it caused a significant inflammatory response. When degeneration among four sealers was evaluated, GuttaFlow had the least irritant effect on rat sciatic nerve. Gencoglu et al. [33] using rats, malonyl aldehyde and glutathione levels in the tissue samples were evaluated. It showed that GuttaFlow exhibited good biocompatibility and acceptable tissue toxicity. The results of our study are in accordance with the results of this study.

Sealapex is widely used RCSs that was produced because of the beneficial biologic effects of calcium hydroxide that it possessed. In this study, among all sealers, the most extensive axon, myelin and epineurium degeneration was seen in Sealapex group. The reason for this extensive degenerative effect was originally thought to be high pH levels that occurred concurrently with the separation of calcium and hydroxyl ions. Huang et al. [34] reported that newly mixed Sealapex had a very high cytotoxicity and they claimed that this toxicity could be related to high pH levels. Studies [35–37] have showed that it can break up easily in tissues and can cause inflammatory reactions.

New calcium silicate-based sealer, Smartpastebio was found to generate an inflammatory response on nerve tissue and severe degeneration of axon and myelin. Zoufan et al. [29] evaluated the cytotoxicity of calcium silicate-based BC Sealer and GuttaFlow when newly mixed and after stiffening; both sealers were found to be highly biocompatible. The results of this study contrast with those of our study. In another study, Mukthar-Fayyad [38] reported that iRoot SP showed mild cytotoxicity in high concentrations, which decreased as the sealer was diluted. Toxic effects with higher concentrations were also linked to its high pH. The calcium hydroxide released during the stiffening reaction of BioAggregate and iRoot SP were reported to possibly be responsible for cell death. In this study, it was suggested that because the sealer was placed directly on the nerve tissue, the high pH of the sealer had destructive effects. Contrary to this study, in other studies [14, 39], it was reported that iRoot SP did not cause severe cytotoxic effects.

Conclusion

All tested RCSs decreased the NCV at different time periods on rat sciatic nerve, but none of them had a severe enough effect to stop the nerve velocity. All sealers induced neurotoxicity as a result of degenerative and inflammatory responses of nerve tissue. Among the sealers evaluated histologically, the most neurotoxic one was Sealapex, and the least toxic was GuttaFlow. However, further animal and clinical studies are necessary to understand the overall behaviours of RCSs for succesful clinical applications.

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