


Comparative safety study of the phenolic constituents of smokebush (*Cotinus coggygria*) leaves

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ABSTRACT

Background: The extract (CPLSB) from the smokebush (*Cotinus coggygria*) leaves is recommended as the endodontic irrigation solution, and its purified fraction contains mainly hydrolyzable tannins (PFTLSB).

Objectives: The purpose of the present study was to define the safety margins as well as the potential irritative and allergenic effects of a polyphenol-containing extract (CPLSB) from the smokebush (*Cotinus coggygria*) leaves.

Methods: The experiments were conducted following the OECD Guidelines for Chemical Testing, followed by probit analysis to precisely establish the median lethal doses (LD50) of CPLSB and PFTLSB.

Results: The LD50 of CPLSB following intraperitoneal and oral dosing in mice was found to be 19 mg/kg and 389 mg/kg, respectively. LD50 of PFTLSB in a similar experiment was 46 mg/kg and 720 mg/kg. Both compositions revealed no signs of toxicity, and the absence of local irritative or allergic reactions was observed as well.

Conclusions: PFTLSB is safer for the intended use because it appears to be almost twice as non-toxic as CPLSB, the maximum oral irrigant dose of which varies between 7 and 14 mg/kg.

Keywords: *Cotinus coggygria*; endodontic irrigation; tannins; toxicity.

BACKGROUND

Smokebush is unique among plant species because it contains high amounts of tannins and practically all flavonoid subclasses.¹ The latter's ability to precipitate and form bonds with proteins is a major determinant of their bioactivity. The wound-healing and antibacterial properties of flavonoids are explained by their interactions with dermal collagen fibers and tannin-induced precipitation of bacterial proteins, respectively.^{2,3}

Pentagalloyl glucose (1,2,3,4,6-penta-O-galloyl- β -D-glucopyranose), the ester of glucose with five moieties of gallic acid, has been one of the first tannins identified from the plant.⁴ Due to the numerous phenolic hydroxy groups in its structure, pentagalloyl glucose exhibits antioxidant, anti-inflammatory, anti-bacterial, anti-diabetic, and anti-cancer activity.

According to several ethnomedical studies,⁵⁻¹⁰ leaves are the most commonly utilized plant component in traditional medicine for skin and mucosal diseases (as extracts, infusions, or fresh juice). The widespread and enduring usage of *C. coggygria* in folk medicine provides a strong foundation for its medicinal use. Data on the pharmacology of *C. coggygria* extracts, however, are still at a very rudimentary level.

The primary goal of endodontic treatment is to eradicate microorganisms from the root canal system. One of the most crucial techniques for efficient root canal disinfection is root canal irrigation. Endodontic research has traditionally focused on discovering procedures or endodontic irrigation that can thoroughly eradicate the bacteria with minimum side effects. Although sodium hypochlorite (NaOCl) is currently the most effective and widely utilized endodontic material, it is a recognized irritant to periapical tissues. In this context, there is still a considerable need for alternative formulations of natural root canal decontaminators.

Natural products have historically been thought to be safer than synthetic ones, but as most of them are complex mixes of organic compounds from several classes, it is necessary to evaluate their safety. It is crucial to know the expected median lethal dosage (LD50) as well as any possible irritative and/or allergic side effects when choosing novel therapeutics.

The current study aimed to determine the safety profile of smokebush (*Cotinus coggygria*) leaf extract.

METHODS

Plant material

High-performance liquid chromatography (HPLC) spectra of smokebush (*Cotinus coggygria*) leaf polyphenols (CPLSB) and



the purified fraction of tannins (PFTLSB) were measured on the Agilent Technologies Infinity 1260 system configured with DAD detector (Agilent Technologies, USA).

Study animals

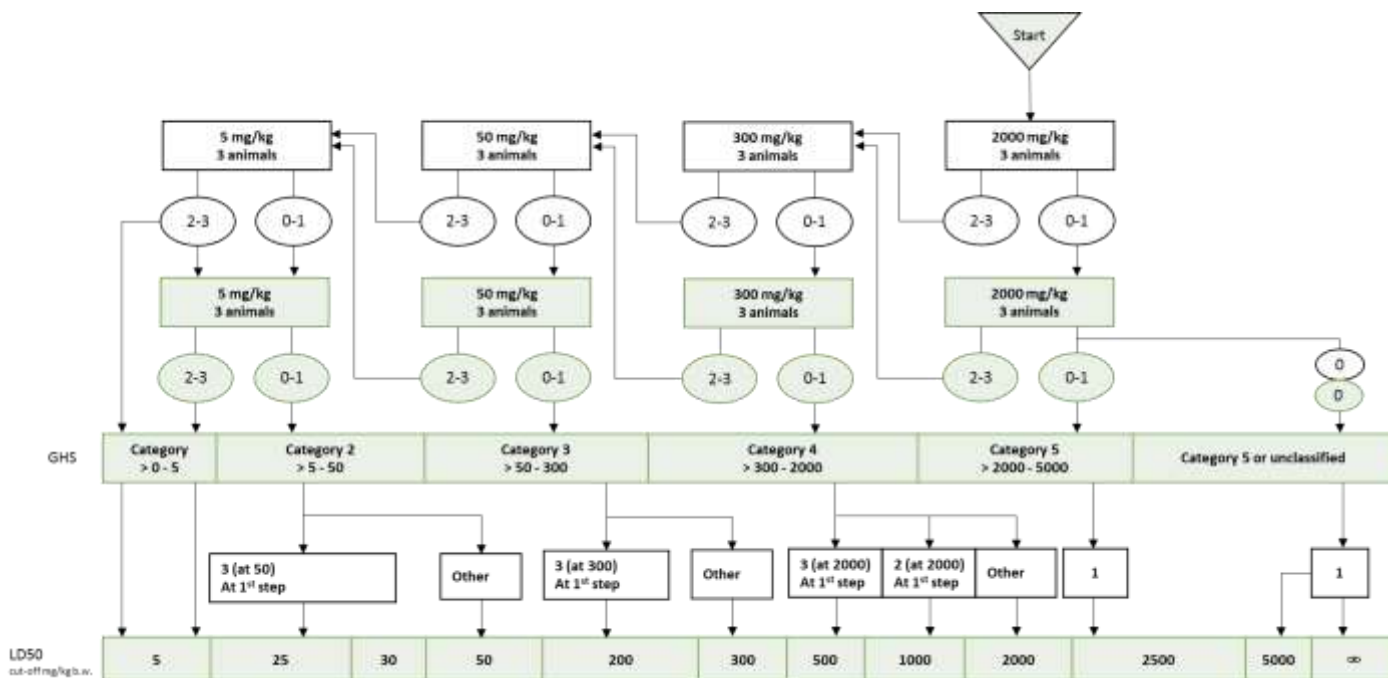
Depending on the type of experiment, the 54 inbred CD1 mice, with a body weight of 272 g, were divided into groups of three or six mice each. The study animals were housed in standard vivarium settings with a 12/12-hour light/dark cycle, a temperature of 20°C, a humidity of 60–70%, daily feedings of standard pelleted food (4 g/mouse), and unlimited access to water. All procedures were carried out under a research protocol that was approved by the Tbilisi State Medical University Ethics Committee on Animal Research (approval number: AP-56-22).^{11,12}

Evaluation of an acute toxicity

The study animals received oral and intraperitoneal injections of the CPLSB and PFTLSB samples following the OECD guideline procedure depicted in Figure 1.¹³ The probit analysis was used to determine the precise value of the median lethal dosage (LD50).¹⁴

After test sample injections the animals were observed for 10, 30 minutes, 1, 2, 4, 8, and 12 hours and then once daily for 7 days to check for mortality and signs of neurotoxicity. At the final stage of the experiment, after CO2 euthanasia of the animals, a macroscopic pathomorphological investigation was conducted.¹⁵

FIGURE 1. Test procedure with a starting dose of 2000 mg/kg body weight and determination of median lethal dose^{13,14}



Abbreviations: GHS, Globally Harmonized Classification System (mg/kg b.w.); LD50, median lethal dosage

Local irritative properties of CPLSB and PFTLSB

In mice of both sexes (n=18), the local irritant impact was investigated.¹⁶ One day before the start of the experiment, 2x2 cm of skin was depilated on the right side of the back of the animals. Then mice were randomly divided into 3 groups (6 animals each) and placed in similar conditions. After 24 hours, a 10% solution of CPLSB and PFTLSB at 0.05 ml/animal (experimental groups) or the same amount of normal saline (control group) was administered topically. The observation was continued for 3 days. On the fourth day, a 10-fold higher dose of the CPLSB and PFTLSB solutions (0.5 ml per animal) was applied, and observation lasted for three more days.

Skin sensitization test

A skin sensitization test¹⁷ with long-term daily applications was used to reveal the possible allergenic properties of CPLSB and PFTLSB in white laboratory mice of both sexes (n=18).

One day before the start of the experiment, the animals were depilated as described above. During the next 14 days, 0.05 ml of CPLSB and PFTLSB 10% aqueous solutions were applied daily to the animal, and the treated skin area was examined. The general condition and behavior of the animals were also assessed daily.

To detect possible immediate or delayed hypersensitivity reactions, a superficial observation of the skin was

conducted the first 30 minutes after the application of the "critical" dose and then at 24, 48, and 72 hours. Animals in the control group were treated with normal saline following the above protocol.

RESULTS

Chemical composition of CPLSB and PFTLSB

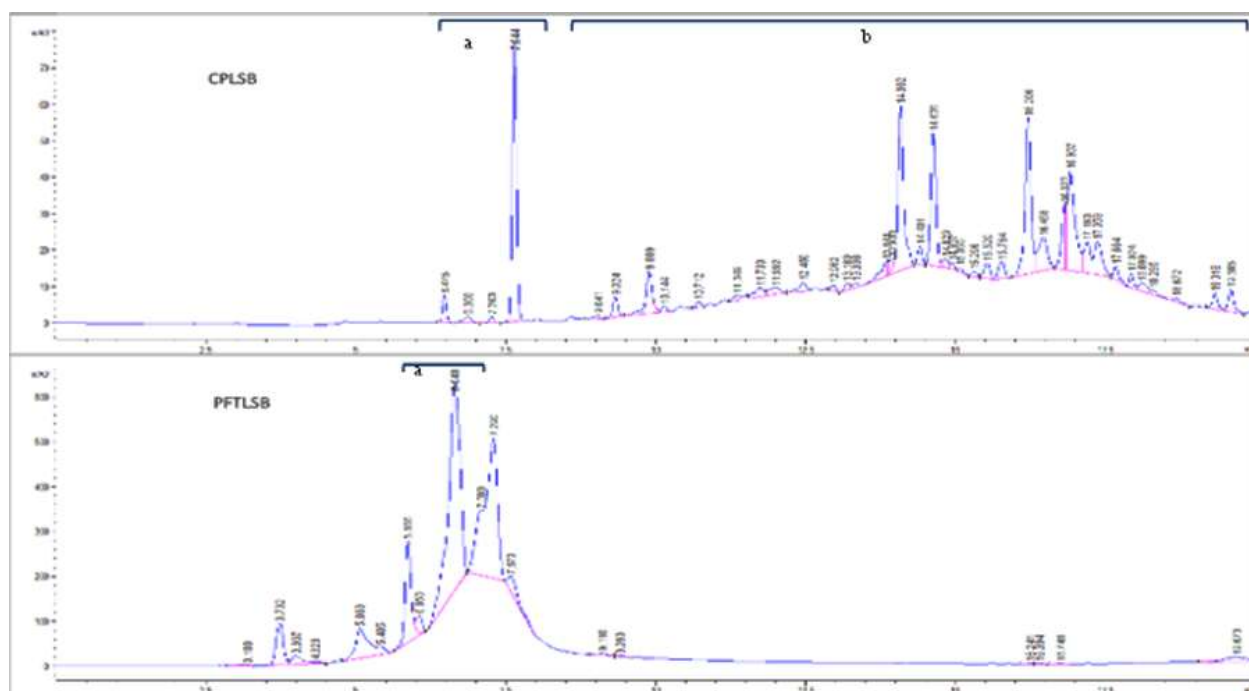
Analysis of the chemical composition of CPLSB and PFTLSB revealed that the latter is free of contains mainly

hydrolyzable tannins (Fig.2) with pentagalloylglucose as the dominant constituent.

Evaluation of acute toxicity

Table 1 shows the probit-analysis-derived LD50 values for CPLSB and PFTLSB. Following the administration of CPLSB and PFTLSB, all remaining animals' general health, food and water intake, and locomotor activity were monitored every day for 7 days.

FIGURE 2. High-performance liquid chromatography/ultra violet (HPLC/UV) spectra of smokebush (*Cotinus coggygia*) leaf polyphenols (CPLSB) and purified fraction of tannins (PFTLSB)



During the observation period, not a single fatal outcome and/or intoxication signs were recorded. The animals did not show any significant changes in terms of movement coordination and vegetative functions. Unconditioned reflexes and reflex reactions to external stimuli were unchanged; breathing and heart rate were kept within the normal range. It should be noted that during the first stage of the experiment all fatal cases were fixed within 24 hours after administration of CPLSB and PFTLSB.

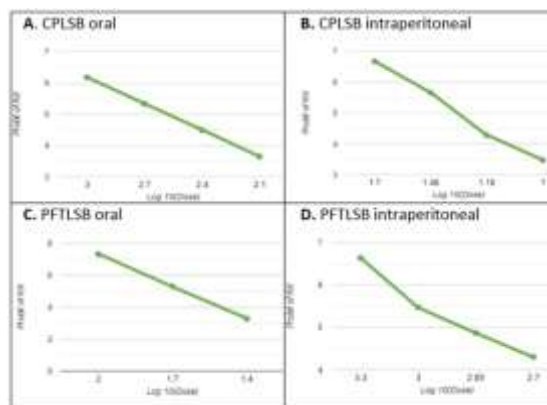
TABLE 1. Acute toxicity of smokebush (*Cotinus coggygia*) leaf polyphenols (CPLSB) and purified fraction of tannins (PFTLSB) in mice

Route of administration	LD 50 (mg/kg)	
	CPLSB	PFTLSB
Oral	389	720
Intraperitoneal	19	46

Abbreviations: CPLSB, smokebush (*Cotinus coggygia*) leaf polyphenols; PFTLSB, smokebush (*Cotinus coggygia*) leaf purified fraction of tannins (PFTLSB)

Figure 3 represents the dose response curves after oral and intraperitoneal administration of CPLSB and purified PFTLSB in mice.

FIGURE 3. Dose response curves after oral and intraperitoneal administration of smokebush (*Cotinus coggygia*) leaf polyphenols (A,B) and purified fraction of tannins (C,D)



Necropsy results

In CPLSB and PFTLSB groups organ-weight-to-body-weight ratios were in normal range and did not differ from that of control animals (Tab.2).

TABLE 2. Organ weights on day 14 after administration of smokebush (*Cotinus coggygria*) leaf polyphenols (CPLSB) and purified fraction of tannins (PFTLSB) in mice

Organ	Absolute organ weight (g)*		
	control	CPLSB	PFTLSB
Liver	1.1374±0.1689	1.1874±0.1886	1.1611±0.1670
Kidneys	0.4343±0.1173	0.4405±0.1077	0.4375±0.0868
Heart	0.1500±0.0195	0.1458±0.0234	0.1465±0.0148
Spleen	0.0785±0.0160	0.0712±0.0138	0.0667±0.0132
	Relative organ weight (% of body weight)*		
Liver	4.3150±0.3040	4.4784±0.5181	4.5116±0.4992
Kidneys	1.6190±0.2128	1.6486±0.2927	1.6864±0.1634
Heart	0.5739±0.0737	0.5515±0.0732	0.5739±0.0740
Spleen	0.3003±0.0581	0.2711±0.0577	0.2621±0.0564

* - all data represented as mean ± standard error of mean (n=6)

Skin: There were no desquamations and no areas of nested hair loss.

Abdominal cavity: the alignment of the internal organs was correct, the mucous membrane was pink, the adipose tissue was well developed, and the serous membrane was clean, smooth, and tough; any defects, exudate, and petechial hemorrhages are not noted.

Heart: no hypertrophy signs, muscles uniformly dense; ventricles normally filled with blood. **Liver:** brownish-indigo, with dense consistency; there were no petechiae under the capsules; no signs of fatty degeneration of hepatocytes fixed.

Lungs: full of air, evenly pink, the surface was glossy, hemorrhages, foci of organic emphysema, and atelectasis were not fixed.

Organs of the gastrointestinal tract: revealed no desquamations, hemorrhages, and/or ulceration of the mucous membrane, as well as accumulations of secretion or mucus, are not revealed.

Kidneys: brownish in color, at the section - glossy, dense, without hemorrhages.

Spleen: dark cherry color, at the section - moderately bloody.

Bladder: full with transparent urine.

Local irritative properties of CPLSB and PFTLSB

The general condition of all animals, movement activity, and food and water consumption did not change during the experiment. No signs of skin irritation were detected either on the day of application or in the subsequent period. Repeated treatment of the same surface with a 10-fold increased dose of neither CPLSB nor PFTLSB did not reveal changes in behavior and general condition. No signs of irritation or contact dermatitis (severe hyperemia, swelling, superficial fissures, punctate bruises, or peeling of the epidermis) were observed.

Skin sensitization test

During the 14-day topical application of a 10% aqueous solution of CPLSB and PFTLSB, as well as within 7 days after its termination, no signs of irritation or dermatosis were observed. Application of the "critical" dose on the treated skin did not cause either immediate allergic reactions within 30 minutes or delayed signs of hypersensitivity that could be manifested by surface redness, swelling, or punctate desquamation.

DISCUSSION

Root canal therapy eliminates bacteria from the root canal system and prevents re-infection. Thus, irrigation of the canal with specific types of solutions throughout the treatment is beneficial.

For many years, sodium hypochlorite in various concentrations has been used most commonly for that purpose; however, concerns have been raised about toxicity and the occasional incidences of pain and/or irritation when higher amounts are used.¹⁸

Alternatives based on natural substances look promising from this perspective, among which is smokebush, a well-known source of polyphenols and tannins. It is asserted that the aqueous extract of *C. coggygria* leaves is effective against bacteria at various doses, but there is no correlation between this antibacterial activity with the concentration of total phenolics.¹⁹⁻²¹ Surprisingly, the majority of published data on compounds produced from *C. coggygria* lacks information on the safety margins for such compounds. Our work attempted to close this informational gap.

The LD50 values of the orally administered PFTLSB and CPLSB were twenty times lower than when given intraperitoneally. The oral PFTLSB was two times less toxic than the CPLSB and has no adverse effects. While individual hypersensitivity to the plant components included in the product cannot be completely ruled out in each case, it is important that PFTLSB must not be characterized by local irritative and sensitizing properties.

Considering that the highest concentration of CPLSB that has been recommended for use as an oral irrigant range between 7 and 14 mg/kg, it can be said that PFTLSB is safer for the intended purpose because it appears to be around twice as non-toxic as CPLSB.

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