ORIGINAL ARTICLE

ANTIMUTAGENIC AND ANTIMICROBIAL EFFECT OF AN EXPERIMENTAL PLANT BASED EXTRACT FOR ENDODONTIC USAGE

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Abstract

An experimental extract based on *Arctium lappa* L. root powder and *Aloe vera* (L.) Burm.f. gel was developed for endodontic usage. Its effect on the microbial viability was previously tested *in vitro*, against bacterial and fungal strains commonly found in the human endodontic environment. The present study focuses on the *in vivo* evaluation of the antimicrobial effect of the experimental plant based extract, using bacteria recovered from the infected root canals of the canine species. We determined the number of colony-forming units before and after using the plant based product. The antimutagenic effect of the experimental extract was determined by detecting mutagenic compounds with the help of the *Salmonella* mutagenicity test (Ames test). Two auxotrophic reference strains were used, *Salmonella typhimurium* TA 98 and *Salmonella typhimurium* TA 100, which carried defective genes, related to the synthesis of histidine. Overall, results showed *in vivo* antimicrobial activity of the experimental extract and also antimutagenic and inhibition effect against the two mutant bacterial strains.

Rezumat

A fost dezvoltat un extract experimental din rădăcină de *Arctium lappa* L. şi *Aloe vera* (L.) Burm.f. gel pentru utilizarea în canalul radicular. Efectul său asupra viabilităţii microbiene a fost testat anterior *in vitro*, asupra tulpinilor bacteriene şi fungice frecvent întâlnite în sistemul endodontic uman. Studiul de faţă se concentrează asupra evaluării *in vivo* a efectului antimicrobian al extractului experimental bazat pe plante, utilizând bacterii recuperate din canalele radiculare infectate ale speciei canine. Am determinat numărul de unităţi formatoare de colonii înainte şi după utilizarea produsului experimental. Efectul antimutagen al extractului a fost determinat prin identificarea compuşilor mutageni cu ajutorul testului de mutagenitate la *Salmonella* (testul Ames). Au fost utilizate două tulpini auxotrofice de referinţă, *Salmonella typhimurium* TA 98 şi *Salmonella typhimurium* TA 100, care au suferit mutaţii anterioare, legate de sinteza histidinei. Rezultatele generale au arătat activitatea antimicrobiană *in vivo* a extractului experimental şi, de asemenea, efectul antimutagenic şi inhibitor asupra celor două tulpini bacteriene mutante.

Keywords: antimicrobial, antimutagenic, Ames test, microorganism, endodontics

Introduction

The development of an ideal root canal disinfectant represents for researchers a continuous challenge. From well-known chemical substances to various plant extracts, all have been studied with the purpose of obtaining an efficient endodontic product, with no side effects.

The plant based extract used in this study was previously tested, along with the identification of different bioactive compounds in its structure. Compounds like chlorogenic acid, lignans and aloe-emodin were proven to be responsible for its antimicrobial effect [19, 23].

In the desire to determine the mechanisms of action of the experimental product, we completed the previous obtained results with *in vivo* findings, taking into consideration that microorganisms organized in biofilms possess different characteristics and behaviours.

The theory of biofilm formation inside the root canals is nowadays self-evident. Persistent endodontic primary and secondary infections are caused by bacteria with behavioural particularities and high affinities for organizing in microbial communities.

Biofilms can be defined as complex communities of microorganisms, attached to a specific surface and protected by a polymeric matrix [21]. In its structure, the infected endodontic system contains all three factors that are essential for the microbial community formation: a solid substrate, fluid channels and the microorganisms [6]. Multiple bacterial and fungal species were previously studied for their capacity to form biofilms inside the root canals. They are able to survive to extreme conditions as a result of the

protective extracellular matrix [13]. In 2012, Ozok A.R. *et al*. reported a number of 606 bacterial species recovered from primary and secondary endodontic infections [16]. Bacteria like *Fusobacterium nucleatum*, *Parvimonas micra*, *Pseudomonas aeruginosa*, *Enterococcus faecalis* and yeasts like *Candida albicans* or *Archaea* species are present in infected root canals [8]. Their distinct behaviour due to their organization in complex microbial communities requires special attention regarding the treatment of root canal infectious disease.

At present, there is no chemical substance to be used in contact with living organisms without a previous mutagenic effect evaluation. The detection of mutagenic compounds in the structure of drugs is currently a matter of major concern. Mutations represent alterations of DNA and RNA sequences that can produce changes in the normal cell proliferation, multiplication, metabolism and physiology. There are two major categories in which genetic mutations can be classified, namely inherited mutations present in all cells of the body and those acquired at some point during the lifetime, present only in specific cells of the organism. Whatever classes they belong to, mutations are closely related to the notion of carcinogenesis.

According to Kowalski's definition, carcinogenicity is the effect of a chemical substance or its metabolic products, which can induce irreversible changes in the normal host cellular scheme. Subsequent, reversible or irreversible cellular alterations, with uncontrolled cellular multiplication appear [9].

In the field of the general medicine and also in dentistry, a variety of drugs are used, that can produce mutations with carcinogenic effects on the organisms.

In endodontics, the most relevant example is sodium hypochlorite. This manages to accomplish most of the conditions regarding the ideal anti-microbial root canal agent. Sodium hypochlorite is commonly used, sometimes along with other substances with selective antimicrobial effect, to irrigate root canals. For example, studies have shown that it is contraindicated to use sodium hypochlorite in combination with chlorhexidine gluconate. A chemical compound with potentially carcinogenic effects [17] is formed when these two products come in contact [4]. However, the use of sodium hypochlorite as an antimicrobial substance in endodontics is nowadays the first option of the dentists, due to the large number and diversity of microorganisms that it manages to combat.

Materials and Methods

Obtaining the experimental extract

The *Arctium lappa* root powder and *Aloe vera* gel extract was developed at the Discipline of Food Science and Technology, University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca, Romania. This consisted in mixing of two separate extracts obtained in advance.

Arctium lappa root powder extract was obtained as follows: the plants were harvested between March and April 2014. After harvesting, they were washed and the roots were separated from the rest of the plant. Inappropriate portions were removed and the remaining roots were ground using the mechanical machining equipment existing in the discipline, in order to obtain a fine consistency of the powder. 100 mg of *Arctium lappa* root powder was introduced into 1 mL of distilled water and mixed for 8 hours. The resulting product was filtered through a 0.45 μm Millipore membrane to eliminate possible impurities. 100 mL of the obtained extract was stored in a test tube for later use.

Aloe vera extract was developed as follows: the leaves of *Aloe vera* were cut at their base and then placed vertically for 6 minutes to drain the dark liquid. Subsequently, the thick outer shell layer was removed and the gel was extracted from the leaf surface. 100 mL of the extracted gel was stored in a test tube for later use.

The *Arctium lappa* root powder and *Aloe vera* gel extract was obtained by mixing 1:1 the two test tubes, containing the *Arctium lappa* root powder extract and the *Aloe vera* extract.

The antimicrobial assay

Isolation of microorganisms. Ten root canals were used as *in vivo* source for the isolation of endodontic microorganisms. These root canals originated from dog teeth (canines and incisors) of five different dogs. The teeth have previously suffered fractures and/or abrasions of the dental crowns and needed endodontic interventions. Pathological tissue was collected before using the experimental extract and subsequently using it, as a Kerr endodontic file (ISO 10) was inserted into the root canal and loaded with infected pulp tissue. It was subsequently removed and placed in a tube containing AMIES transport medium.

In order to test the action of the experimental extract, root canals were filled with the test product, which was allowed to act for 10 minutes. After removing it by suction, a Kerr endodontic file (ISO 10) was inserted into the root canal and loaded with the remaining contents.

The endodontic file was inserted into a tube, containing AMIES transport medium. The procedure was repeated for each root canal. All tubes containing the endodontic files were sealed and transported to the microbiology laboratory. The root canals were then filled with calibrated gutta-percha cones and sealing paste.

The teeth were then reconstructed coronary.

Culture conditions of microorgansims. We have inoculated the samples on Columbia Agar culture medium, enriched with blood for bacteria and on Agar Sabouraud for yeasts. Culture media were purchased from Oxoid, UK. We applied the endodontic file on a 2 cm^2 area of the culture medium and then spread the contents on the entire surface of the plate. We incubated the culture media as follows: for 24 hours at 37°C for bacteria and for 5 days at 28°C for yeasts. After incubation, we examined the morphological and cultural characteristics of plate-derived colonies. We later identified the microorganisms as Gram-positive or Gram-negative; we determined their form and group affiliation (cocci, bacilli, streptococci, streptobacilli) and we counted the colonies both before and after using the experimental extract.

The antimutagenic assay

The mutagenic potential was assessed using the Ames standard test [11, 12]. In order to avoid an erroneous result due to the antimicrobial effect of the samples against the test strains, a preliminary experiment was performed in which a dose interval appropriate to the Ames test was determined.

The samples were prepared as follows: 0.1 mL *S. typhimurium* TA98 and *S. typhimurium* TA100 were cultured for 10 hours. We obtained 108 cells/mL of each strain. These were mixed with 0.1 mL extract (5 mg/plate), 0.1 mL phosphate buffer (0.2 M, pH 7.4) and 0.5 mL S9 mix or phosphate buffer. Serial dilutions were performed immediately with phosphate buffer and the aliquot was then mixed with 12 mL nutrient agar. After incubation at 37°C for 48 hours, the number of initial colonies was determined.

Effective testing of the antimutagenic effect was carried out as follows: we mixed the previously obtained product with 0.2 mL of histidine-biotin, given that the strains had previously undergone a mutation in the gene responsible for the production of histidine. The mixture was added to a tube containing 2 mL Top agar, which was then slowly centrifuged. We obtained a new blend that we poured on a MA (minimal agar) plate. This plate was characterized by its content in the minimum of nutrients required for the development of microorganisms.

Generally, a substance with a mutagenic effect, in the presence of histidine, will produce to the mutant strain an inverse mutation at the level of the gene in which it had undergone the previous mutation. Thus, the auxotrophic strain of *S. typhimurium*, by now incapable of producing histidine will become, due to the reverse mutation, capable of producing histidine. It will bear the name of "revertant" bacterium. This will multiply on the MA medium, from which the minimum amount of histidine has already been used in the initial bacterial growth process. The ability of a revertant bacterial strain to multiply on a non-histidine culture medium, in the presence of the test substance, proves that this substance possesses a mutagenic character.

Since some groups of substances exhibit minimal mutagenic effect in the absence of exogenous metabolic activation, we have added S9, the rat liver homogenate, to provide the intake of enzymes that cause the transformation of potentially carcinogenic compounds into active carcinogen compounds. In order to monitor the effect of rat hepatic homogeneity, we tested each extract with and without S9 mix, the plates being poured in triplicate for each dose of extract.

The preparation of the diagnostic substance was carried out as follows: 2-anthramine (2-AA) (5 μg/plate, 2 μg/plate, 20 μg/plate) was dissolved in DMSO (dimethyl-sulfoxide) and introduced into the strain-based mixture, phosphate buffer and S9 mix or phosphate buffer. After incubation at 37°C for 48 hours, revertant bacterial colonies were counted. For negative control of the mutagenic effect, we used the original mixture in the absence of any test extract, treated with DMSO. After incubation at 37°C for 48 hours, revertant bacterial colonies were counted.

A compound was considered to be mutagen if there was a square-rise of revertant colonies (*versus* negative control) or if there was a dose-dependent increase in the number of revertant colonies for one or more strains. The toxicity effect was confirmed when the amount of microorganisms *per* plate was lower than that of the test compound (without added compounds). A schematic approach of the AMES test is shown in Figure 1.

Statistical analysis

We calculated the statistical significance of the differences between the number of colonies determined prior using the experimental product and after using it with the paired Student t test. We set the limit of statistical significance at $p < 0.05$.

The mutagenic effect of the positive control substance was defined as 0% inhibition. We calculated the mutagenic effect for each extract using the following formula [15]:

$$\text{Inhibition} = [1 - T/M] \times 100,$$

where T represents the number of revertants *per* plate in the presence of positive control and extract and M is the number of revertants *per* plate in the presence of control substances (in the absence of the extract). The test was performed in duplicate, with three sub-determinations for each case. All data was expressed according to standard deviations.

For the variance analysis we used the Mann-Whitney test. We set the limit of statistical significance at $p < 0.05$. Data were analysed using SPSS, V22 (SPSS, Inc., Chicago, IL, USA). Data were described using arithmetic mean, standard deviation, median and percentiles of 25 and 75 respectively.

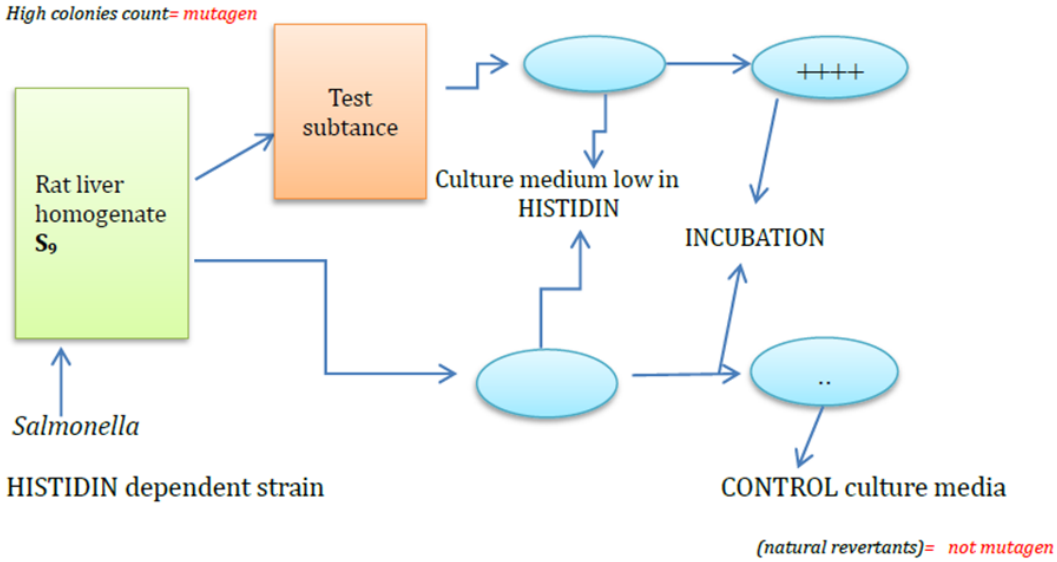

Figure 1.
Stages of the AMES test, for evaluation of the mutagen effect of a tested substance

Results and Discussion

The antimicrobial assay

We obtained the results of the antimicrobial assay by counting the colony-forming units before and after use of the experimental product. The results are shown in Table I. Statistical analysis of the data, showed $p < 0.05$, rejecting H0, by which we assumed that there was no difference between the number of colonies determined prior to the use of the experimental product and the number of colonies resulted after its use.

Table I

Number of colonies, expressed in CFU/cm^2 before and after using the experimental plant based extract

Sample number	Initial number of colonies (CFU/cm^2)	Number of colonies (CFU/cm^2) after using the experimental extract
1	60	15
2	23	8
3	41	12
4	0	0
5	0	0
6	29	16
7	16	7
8	52	24
9	37	20
10	21	13

The synthesis of the values obtained in the t test is shown both in Table II.

Table II

Values obtained with the Student's t test

	Variable 1	Variable 2
Mean	27.9	11.5
Variance	404.1	62.2777
Observations	10	10
Pearson Correlation	0.8436	
H.M.D	0	
df	12	
t Stat	2.4014	
p (T ≤ t) one-tail	0.0167	
t Critical one-tail	1.7822	
p (T ≤ t) two-tail	0.0334	
t Critical two-tail	2.1788	

The antimutagenic assay

At the Ames test we obtained following results: the number of bacterial strains of *S. typhimurium* TA98 determined in the presence of *Arctium lappa* extract and in the absence of S9 mix was 40 CFU/plate. In the absence of S9 mix and also in relation to *S. typhimurium* TA98, we determined for *Aloe vera* extracts 42 CFU/plate. In the case of *Arctium lappa* and *Aloe vera* extract we determined in the absence of S9 mix 40 CFU/plate. For the negative control substance of the mutagenic effect we determined 49 CFU/plate of *S. typhimurium* TA98, in the absence of S9 mix. In relation to this, the average number of bacterial colonies determined in case of all plant extracts was lower than the negative control and we can state that in the presence of *S. typhimurium*

TA98 and in the absence of S9 mix, no herbal extract exerted a mutagenic effect.

Upon addition of the S9 mix, *S. typhimurium* TA98 gave rise to the following determinations of colony-forming units: for *Arctium lappa* extract 33 CFU/plate, *Aloe vera* extract 31 CFU/plate and *Arctium lappa* and *Aloe vera* extract 31 CFU/plate. In case of the negative control substance we determined 32 CFU/plate for *S. typhimurium* TA98, in the presence of S9 mix. In the case of the positive control of the mutagenic effect we determined 763 CFU/plate for *S. typhimurium* TA98 in the presence of S9 mix. In relation to this, the number of bacterial colonies determined in case of all plant extracts was lower than in case of the positive control and similar to the negative control determinations. We can assert that in the presence of *S. typhimurium* TA98 and S9 mix, no herbal extract exerted a mutagenic effect (Figure 2).

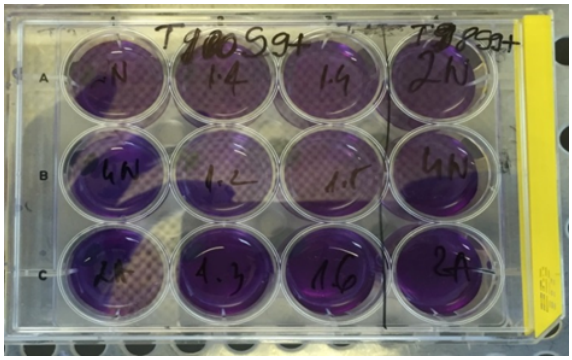

Figure 2.
Determination of mutagenicity of *Arctium lappa* and *Aloe vera* extract in the presence of *S. Typhimurium* TA98

The number of bacterial strains of *S. typhimurium* TA100 determined in the presence of *Arctium lappa* extract and in the absence of S9 mix was 110 CFU/plate. Also in the absence of S9 mix and in relation to *S. typhimurium* TA100, we determined 100 CFU/plate in case of *Aloe vera* extract. In case of *Arctium lappa* and *Aloe vera* extract we determined in the absence of S9 mix 107 CFU/plate. For the negative control substance of the mutagenic effect we determined 110 CFU/plate for *S. typhimurium* TA100, in the absence of S9 mix. In relation to this, the average number of bacterial colonies determined in case of all plant extracts was lower than for the negative control and we can state that in the presence of *S. typhimurium* TA100 and in the absence of S9 mix, no herbal extract exerted a mutagenic effect.

Upon addition of the S9 mix, *S. typhimurium* TA100 gave rise to the following determinations of colony-forming units: for *Arctium lappa* extract 126 CFU/plate, for *Aloe vera* extract 117 CFU/plate and for *Arctium lappa* and *Aloe vera* extract 125 CFU/plate. For the negative control substance of the mutagenic effect, we determined 124 CFU/plate in the presence of the S9 mix. For the positive control of the mutagenic effect, we determined the 885 CFU/plate in the presence of S9 mix. In relation to this, the number of bacterial colonies determined in case of all plant extracts was lower than in case of the positive control and similar to the negative control determinations. We can assert that in the presence of *S. typhimurium* TA100 and S9 mix, no herbal extract exerted a mutagenic effect (Figure 3).

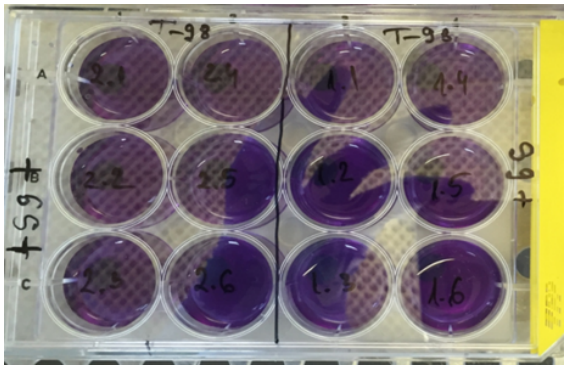

Figure 3.
Determination of mutagenicity of *Arctium lappa* and *Aloe vera* extract in the presence of *S. Typhimurium* TA100

The results in the form of averages of the determined colony forming units are shown in Table III.

Table III

Average of CFU determinations for the tested substances, to *S. typhimurium* TA98 and *S. typhimurium* TA100, with and without S9 mix

Tested substances	**Number of colonies (CFU/plate)**			
	TA 98		TA100	
	S9-	S9+	S9-	S9+
Negative control substance	49	32	110	124
***Arctium lappa* extract**	40	33	110	126
***Aloe vera* extract**	42	31	100	117
***Arctium lappa* and *Aloe vera* extract**	40	32	107	125
Positive control substance (0.005)	-	763	-	-
Positive control substance (0.002)	-	-	-	885

We assumed that the antimutagenic activity of the positive control substance represents 0% inhibition of the mutant strains. For the tested extracts we thus obtained for *S. typhimurium* TA98 a 96% inhibition, in the presence of each extract. In case of *S. typhimurium* TA100, in the presence of *Arctium lappa* extract, we obtained an inhibition of 86%. In the presence of *Aloe vera* extract we obtained an inhibition of 87% and in the case of *Arctium lappa* and *Aloe vera* extract we obtained an inhibition of 86%.

The mean percent inhibition rates for each test substance are shown in Table IV.

Table IV

The antimutagenic effect of tested substances, expressed in inhibition percentage

Tested substance (mg/plate)	Inhibition	
	TA 98	TA100
Negative control substance	0	0
Mutagen + *Arctium lappa* extract	96	86
Mutagen + *Aloe vera* extract	96	87
Mutagen + *Arctium lappa and Aloe vera* extract	96	86

Values obtained at the Mann-Whitney test showed $p < 0.05$ for each strain tested. This rejects H0, by which we assumed that all the test groups would demonstrate the same mutagenic effect for the reference strains, by obtaining the same values of the resulting CFUs. Thus, the clinical results are complemented by the results of the statistical tests, which reveal a statistically significant difference between the studied groups. The values obtained with the Mann-Whitney test are shown in Tables V-VIII.

Table V

Values obtained with Mann-Whitney test for *S. typhimurium* TA98

	Rank average	**Rank Sum**	**Z value**	**p value**
S9-	6.50	26.00	-1.482	0.046
S9+	3.80	19.00		

Table VI

Values obtained with Mann-Whitney test for *S. typhimurium* TA100

	Rank average	**Rank Sum**	**Z value**	**p value**
S9-	2.5	10	-2.460	0.016
S9+	6.5	26		

Table VII

Values obtained with Mann-Whitney without S9 mix

	Rank average	**Rank Sum**	**Z value**	**p value**
S. typhimurium TA98	6.50	26.00	-2.337	0.029
S. typhimurium TA100	3.80	19.00		

Table VIII

Values obtained with Mann-Whitney with S9 mix

	Rank average	**Rank sum**	**Z value**	**p value**
S. typhimurium TA98	3.80	19.00	-1.781	0.075
S. typhimurium TA100	7.20	36.00		

In the context of the current studies, antimicrobial and antimutagenic properties of a substance represent key characteristics for an efficient endodontic disinfectant. They set the premises for a safe and effective usage.

The results of *in vivo* testing of the experimental product revealed an important antimicrobial activity against bacterial strains identified in the canine species. However, the limitations of the study consisted in the difficulty of raising awareness among dog owners of the need for endodontic treatment in animals. The anaesthetic procedures required for the treatment have led to the rejection of this variant for their animals and implicitly to the reduction of the number of cases present in the study.

Another impediment of the study was the difficulty of interpreting subjective signs, causing errors in establishing the diagnosis. Thus, the patient who presented the entire abraded frontal dental group was found to suffer from pulp necrosis in 3 teeth of a total of 5, requiring endodontic treatment. The micro-biological examination revealed for the other two teeth a negative microbial load. Subsequently, we established the diagnosis of pulpal inflammation and not pulpal necrosis.

Although bacterial species in the root canals of dogs can differ from the root canals of humans, they present similarities in the ability to organize in bio-films and to establish microbial interactions. Dogs'

teeth have been therefore used in numerous studies as experimental models [7].

Most relevant examples of microorganisms that are present both in human and canine root canals are represented by *Enterococcus faecalis* and *Candida albicans*. Along with other microorganisms, they present various virulence factors, responsible for their pathogenicity.

E. faecalis is the most resistant microorganism isolated not only from primary infections, but also from root canal filled teeth [22]. It possesses virulence factors such as serine protease and gelatinase; enterococci surface proteins, collagen adhesion proteins and polysaccharides [24]. These are responsible for distinct virulence mechanisms and resistance processes against antibiotics, such as penicillin, erythromycin, ciprofloxacin or vancomycin [1]. Amoxicillin + clavulanic acid was proven to be the most efficient antibiotic against *E. faecalis* and also against other tested endodontic bacterial strains [1].

C. albicans is the most present fungal species in the infected endodontic systems [18]. It has the capacity to adhere to the root canal walls and to survive at low pH, due to the capacity to change its morphology, from yeast to hyphal form [14]. It is able to coexist with a number of bacteria in specific root canal biofilms and together with *E. faecalis* it is able to withstand starvation processes [5].

Even if *in vitro* disc diffusion methods represent valuable techniques, that bring important antimicrobial information, it is mandatory to complete the obtained results with *in vivo* findings, in order to outline, as accurately as possible, the mechanisms of action of the tested substance. The results of the present study bring therefore new information regarding the antimicrobial efficiency of *Arctium lappa* and *Aloe vera* extract, by underlining its action against endodontic microorganisms in their natural environment.

The importance of assessing the mutagenic effect of the *Arctium lappa* and *Aloe vera* experimental extract consists in the fact that further research would not have been possible if its mutagenic action was proven. Furthermore, by using Ames test, an *in vitro* method based on bacterial cell systems, no laboratory animal was sacrificed.

The use of an effective product that does not exhibit negative effects on the tissues it comes in contact with, is a necessary condition in the development of any experimental substance.

A multitude of antibiotic-based substances have been created for their topical use in the root canal. Even if some do not show carcinogenic effects, there are still unpleasant consequences that may arise from their use.

In endodontics, the effects of antimicrobial substances used in the root canal are of greater importance in paediatric dentistry. Teeth with open apexes incompletely formed or deciduous teeth represent cases where the undesirable effects of antimicrobial agents are numerous. Recent studies have made a review of drug substances used directly in the root canal in paediatric endodontics and concluded that many of them can exhibit potentially carcinogenic effects [3]. The product called Pulpomixine® contains dexamethasone acetate, polymyxin B sulphate and framycetin sulphate. Indications of its use consist of direct and indirect pulp capping and acute pulpitis with the recent exposure of the pulp chamber (BANSAL, 2014). The polymyxin class is used in general medicine to solve cases of infections with multiple bacterial resistant strains, such as *Pseudomonas aeruginosa*, *Acinetobacter baumanii*, *Escherichia coli* or *Klebsiella pneumoniae*. Their long-term use has, however, led to the occurrence of mutations in the bacterial strains and implicitly to the development of the resistance phenomenon [2].

Among the chemical substances used in the irrigation of the root canals, sodium hypochlorite occupies by far the first place in the preferences of the practitioners. It has the ability to dissolve necrotic tissues and to eliminate a wide variety of microorganisms. However, recent studies have shown that the formation of additional compounds, such as chloroform, benzoic aldehyde, dichloromethylbenzene or hexachloroethane, occurs when this substance comes in contact with the pulp tissue or dentin. Of these products, some can cause significant adverse effects on the health of the patient and of the physician. Generic organochlorine compounds have been identified for all sodium hypochlorite concentrations commonly used in irrigation of root canals (0.5%, 2.5%, 5.25%) [20]. Organoclorines are substances frequently identified in pesticide products, studies demonstrating a close link between them and various forms of cancers [10].

Given the current status of cytotoxicity and antimicrobial efficiency of products for endodontic usage, there is a constant need for development of new substances that work effectively as antimicrobial agents, without side effects. Due to their antibacterial potentials and no mutagen effects, plant based products like *Arctium lappa* and *Aloe vera* extract are more preferred, when compared to chemical substances.

Conclusions

The experimental extract showed *in vivo* antimicrobial activity against microorganisms in the root canals of dogs. It also did not exhibit mutagenic effects on the auxotrophic strains *S. typhimurium* TA98 and *S. typhimurium* TA100. The obtained results may suggest that the extract will not have mutagenic effects on patients either.

Further, well-conducted studies, are needed in order to determine the mechanisms of action of the

experimental extract in *vivo,* in the infected root canal of humans.

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