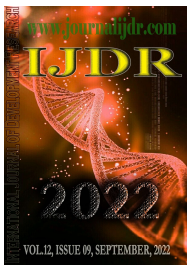
ISSN: 2230-9926

*Available online at http://www.journalijdr.com*

IJDR

*International Journal of Development Research*
*Vol. 12, Issue, 09, pp. 58622-58626, September, 2022*
https://doi.org/10.37118/ijdr.25268.09.2022




RESEARCH ARTICLE OPEN ACCESS

# HOMEOPHATIC EFFECT OF *CALCAREA CARBONICA*, *CALCAREA PHOSPHORICA* AND *CALCAREA FLUORICA* IN INORGANIC COMPOSITION OF DENTAL PULP IN RATS

**Nardy RO[1,*] Amaral, LA[2], Nunes, A.M[3]; Barroso, L.S[4].; Cataldo, D[5]; Motta, V C[6]; Mourão, L. C. S[7] and Leite MF[8]**

[1]Phd, Msc, DDs - Rosy de Oliveira. Nardy, Department of Endodontics, School of Dentistry. University of Volta Redonda-Rio de Janeiro-Brazil (UNIFOA); Brazilian Association of Recycling and Assistance in Homeopathy(ABRAH); [2]DDs Luciano Abbá do Amaral, Department of Endodontics, School of Dentistry. University of Volta Redonda - Rio de Janeiro - Brazil (UNIFOA); [3]PhD, MSc, DDS –Adriana Marques Nunes - Department of Endodontics, School of Dentistry. University of Volta Redonda - Rio de Janeiro-Brazil (UNIFOA); [4]PhD, MSc, DDS-Leonardo dos Santos Barroso Department of Endodontics, School of Dentistry. University of Volta Redonda-Rio de Janeiro-Brazil (UNIFOA); [5]DDs, Dionísia Matos Cataldo-Association of Recycling and Assistance in Homeopathy (ABRAH) Rio de Janeiro, Brazil; [6]Msc, DDs Vicente Canuto Motta-Brazilian Association of Recycling and Assistance in Homeopathy (ABRAH) Rio de Janeiro, Brazil; [7]PhD, MSc, DDS-Leila Cristina dos Santos Mourão, Department of Periodontology, School of Dentistry, Universidade Veiga de Almeida (UVA), Rio de Janeiro, Brazil. Brazilian Association of Recycling and Assistance in Homeopathy (ABRAH) Rio de Janeiro, Brazil; [8]Phd Msc, DDs-Mariana Ferreira Leite, Departament of Dentistry - University Cruzeiro do Sul (UnicSul)-São Paulo – Brazil

## ARTICLE INFO

***Article History:***
Received 08th July, 2022
Received in revised form
21st July, 2022
Accepted 11th August, 2022
Published online 20th September, 2022

***Key Words:***

Homeopathy, Dental Pulp, Inorganic Composition.

****Corresponding author: Nardy RO***

## ABSTRACT

***Introduction:*** Inorganic composition of pulp tissue is related to its regeneration potential in tooth mineralization processes. ***Objetive:*** The objective of the study was to evaluate the effect of administering Calcarea carbonica 6 CH, Calcarea phosphorica 6CH and Calcarea fluorica 6CH in inorganic composition of dental pulp. ***Methodology:*** Wistar rats (n=40) were divided in groups according to the experimented medication: GI-control group, GII – Calcarea carbonica 6CH, GIII-Calcarea phosporica 6CH, GIV – Calcarea fluorica 6CH and GV homeopathic formula composed by all of them. The animals received 3 drops of medication daily, corresponding to the group, during 21 days. The animals were sacrificed on the 22nd day and the incisor dental pulp was removed. Ca, P and K concentrations were determined by optical emission spectrometry (ICP-OES). The results were subjected to Analysis of Variance and Tukey´s Post Hoc Test ($p \leq 0,05$). ***Results:*** Groups GII and GV presented an increase in the concentration of Ca (80 and 88% respectively) and P (54 and 58% respectively) compared to the control group (GI) ($p \leq 0,05$). Groups GIII, GIV and GV presented an increase in concentration of K (17, 34 and 46% respectively) compared to the GI group ($p \leq 0,05$). Treatment with Calcarea carbonica, phosphorica and fluorica increased the animals' body weight in comparison with the control group. ($p \leq 0,05$). ***Conclusion:*** Calcarea carbonica 6CH and the homeopathic formula composed of the three Calcareas increase the availability of calcium, phosphorus and potassium in pulp, which can contribute positively to dental mineralization processes. Calcareas phosphorica and fluorica 6CH, as well as their combination, increase the availability of potassium in the pulp tissue.

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**Citation:** ***Nardy RO., Amaral, LA., Nunes, A.M., Barroso, L.S et al.*** "Homeophatic effect of calcarea carbonica, calcarea phosphorica and calcarea fluorica in inorganic composition of dental pulp in rats. *", International Journal of Development Research*, 12, (09), 58622-58626.

# INTRODUCTION

The literature records comparative studies between allopathic and homeopathic medicines. Among these studies, the evaluation of systemic medications that induce bone neoformation shows favorable results for homeopathy[1,2,3] Dental pulp has regenerative potential; it has stem cells inside it that are capable of differentiating into specialized cells. Odontoblasts are responsible for dentin production and demineralization[3,4,5,6,] Dental pulp is highly vascularized and composed of myelinated and unmyelinated nerve bundles. This property correlates with the other two main functions of the dental pulp, which must provide nutrition and mineralization of dentin, influenced by its inorganic constitution[7] The literature reports the

importance of maintaining and regenerating pulp vitality for long-term tooth viability. Complete or fragmented remaining vital pulp tissues allow regeneration and complete pulp revascularization, when submitted to adequate pulp therapy [8,9] Self-regulation's function is to keep the internal constants in constant variation according to the circumstance, which is equivalent to the state of health, while the opposite is equivalent to the state of disease. Homeostasis or Self-regulation, result from stimuli in action receptors, represented by nerve endings, membrane receptors and others, all of electromagnetic character, since even the chemical ones, in the last analysis, are stimulated by photon exchange. All this movement has the purpose of creating physiological action programs and stimulating effector organs that are responsible for the stabilizing action itself or self-regulation[9,10.] In this dynamic conception, the immaterial and electromagnetic homeopathic stimulus has the function of stimulating in a similar way the Self-regulation system towards the search for equilibrium or, in other words, health[11,12]. Carillo's Complex Systems Model proposes that the self-regulatory function is related to five other elements, besides the Structure or Organism. Among them, Cognition is fundamental to the understanding of the healing phenomenon through Homeopathic treatment. Inadequate Action Results, which represent the deficiency of Self-regulation and, consequently, the state of disease, can be due to the absence or deficiency of Action Programs for the accomplishment of certain tasks. In the case of the maintenance of ideal salivary Ca++ levels, the homeopathic medicine, capable of provoking an instability similar to the disease it intends to cure, stimulates the cognitive function of the system, correcting or increasing the Action Programs necessary for the cure of the disease, balancing salivary Ca++ levels. In this sense, it can be said that Homeopathy is an essentially cognitive therapy, the only way to treat diseases called chronic or even incurable by the allopathic method[13, 14].

The presence of calcium and phosphorus ions in dental structures increases the surface hardness of the enamel of newly erupted teeth and has the purpose of maintaining vascularization, sensitive synaptic transmissions and, above all, maintain dental formation and remineralization processes (ALVES; SEVERI, 2016). The processes of Homeostasis or Self-regulation, as it is called by Anokhin's Physiology, (2013)[10]are composed of five elements: *the Result of Action, Receiver of the Result of Action, Gauging Device of the Result of Action; the Organizing Device of Action; Effector Organs of Action*. Each and every movement made by *Self-regulation* to maintain *Homeostasis* will take place in an action receptor structure, where the stimuli coming from these receptors will be evaluated and transformed according to the needs of the system itself in face of the circumstances presented. *The Action Organizing Device* is responsible for triggering or creating programs for the correction of inadequate *Action results*, where the *Effector Organs* are responsible for the stabilizing action itself. The aim of this study was to evaluate the levels of calcium, phosphorus, potassium and sodium in dental pulp of rats treated by Calcareacarbonica, Calcareaphosphorica and Calcareafluorica homeopathic medicines that act directly on organic metabolism.

# METHODOLOGY

Experimental study of the laboratory trial type, submitted to the Research Ethics Committee of the University Center of Volta Redonda - UniFOA, approved under protocol number 248/052012, according to the ethical rules for scientific procedures and animal studies adopted by the Brazilian College of Animal Experimentation (COBEA). We used 40 adult male Wistar rats (225.6 ± 17.1 g), from the vivarium of the Centro de Criação de Animais de Laboratório/ FIOCRUZ-BRASIL. Housed in Complete Polypropylene Box cages 41 x 34 x 16 cm - Insight® (04 animals / cage) under standard laboratory conditions: 12h light / dark cycle; lights on at 07:00; 22 ± 2° C and ad libitum access to water and Purina rat chow. The animals were divided into 05 groups: control (GI) and experimental (GII, GIII, GIV and GV) according to the medicine tried: *G I - Control:* Rats (n=8) placebo composed of 30% alcoholic solution for homeopathic medicines . *G II - Calcareacarbonica 6CH:* Rats (n=8) treated with *Calcareacarbonica 6CH*. *G III - Calcareaphosphorica 6CH:* Mice (n=8) treated with *Calcareaphosphorica 6CH*. *G IV - Calcareafluorica 6CH:* Mice (n=8) treated with *Calcareafluorica 6CH*. *G V - Calcarea compound:* Rats (n=8) treated with *Calcareacarbonica 6CH+ Calcareaphosphorica 6CH + Calcareafluorica 6CH* homeopathic compound formulation. These animals received a dose of 03 drops of medication from their respective group, daily, between 7am and 8am, which were dispensed into the mouth of each rat with the aid of a Pasteur pipette. During the period between days 1 and 21 of the experiment, they were euthanized on day 22 by decapitation.

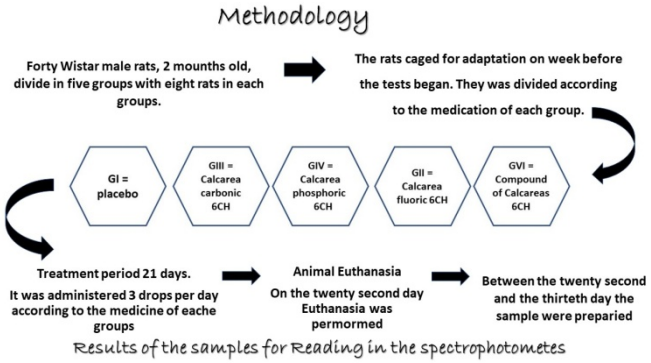


**Figure 1. Methodology**

Dental pulps from the upper and lower incisors were removed using Hedstrom 10, 15, 20 and 25 endodontic files, according to the size of the root canal. Each sample represented an aliquot of four incisor pulps from each mouse to obtain an adequate amount of tissue (30 ± 10 mg). After removal, they were immediately frozen and stored at -80° C until the time of analysis. For the analysis, the pulp tissues underwent a tissue digestion process in a solution of 1ml of nitric acid + 1ml of 30% H2O2, submitted to a water bath in an incubator at 50° C for 10 min. Subsequently, the crude homogenate of the digested sample was filtered on filter paper with the help of 50 mm sodium phosphate buffer solution, pH 7.4. The filtered homogenate was used for further analysis. The determination of inorganic components was by inductively coupled plasma - optical emission spectrometer (ICP-OES), for the dosage of calcium, phosphorus, potassium, sodium. 720 ICP-OES from Agilent (Agilent 720 series ICP optical emission spectrometry).

# RESULTS

Data presented as mean ± standard error of the mean (SEM). The Anderson-Darling test was applied to evaluate the frequency distribution of the data. After checking the normality of the data distribution, the biochemical parameters of the groups studied were compared by analysis of variance and Tukey's multiple comparisons test. The significance level adopted was 5% ($p < 0.05$). Mean values and standard deviation for calcium concentration (mEq/mg tissue) in the dental pulp of rats in the control group and experimental groups evaluated in the study are presented in graph 1 and table 1. The group treated with Calcareacarbonica (GII) showed a 79% increase in calcium concentration (59.98 ± 14.56) in the pulp tissue when compared to the control group treated with placebo drug (33.44 ± 12.71), a result considered statistically significant with $p \leq 0.05$. The combined formula of the three calcareas (GV) showed a significant increase in calcium concentration (96.64 ± 65.98) in the pulp tissue when compared to all other groups evaluated (GI - 33.44 ± 12.71; GII - 59.98 ± 14.56; GIII - 36.34 ± 12.17; GIV - 40.72 ± 14.19, 188%, 61%, 160%, 130%, respectively) ($p \leq 0.05$). Phosphoric and fluoric limestones showed no significant effects on calcium concentration in the pulp tissue compared to the control group.Graph 1 - Calcium concentration (mEq/mg tissue) in dental pulp of rats in the groups: control (GI), treated with *Calcareacarbonica (GII)*, treated with *Calcareaphosphorica (GIII)*, treated with *Calcarea Fluorica (GIV)*

and treated with the combination of *Calcareacarbonica, Calcareaphosphorica and Calcarea Fluorica (GV*). (*) (#) Statistically significant difference compared to group (GI) and (GV), respectively, p≤0.05. Mean values and standard deviation for phosphorus concentration (mEq/mg tissue) in dental pulp of rats from control (GI) and experimental (GII, GIII, GIV, GV) groups are represented in figure 10 and table 1. By comparing the groups among themselves, a significant increase in calcium concentration in dental pulp was observed only in the groups that received treatment with *GII - Calcareacarbonica* (54%) (353.05 ± 79.04) and with GV - association between Calcareas (58%) (361.87 ± 138.93) compared to the control group (228.31 ± 59.25) (p≤0.05). No differences were observed between the GII and GV groups.

Graph 2 - Phosphorus concentration (mEq/mg tissue) in dental pulp of rats from the groups: control (GI), treated with *Calcareacarbonica (GII)*, treated with *Calcareaphosphorica (GIII)*, treated with *Calcarea Fluorica* (GIV) and treated with the combination of *Calcareacarbonica, Calcareaphosphorica and Calcarea Fluorica* (GV). (*) Statistically significant difference compared to group (GI), p≤0.05. The mean values and standard deviation of potassium concentration (mEq/mg tissue) in dental pulp of rats from control (GI) and experimental (GII, GIII, GIV, GV) groups are represented in figure 11 and table 1. Only groups GIV (9.34 ± 1.57) and GV (10.15 ± 0.98) showed significant differences in potassium concentration when compared to the control group (6.95 ± 1.38) (p≤0.05). *Calcareafluorica* and the combination of Calcareas showed an increase in potassium concentration of 34% and 46% compared to the control group. *Calcareacarbonica and phosphorica*showed no statistically significant differences compared to the control group Graph 3 - Potassium concentration (mEq/mg tissue) in dental pulp of rats from the groups: control (GI), treated with *Calcareacarbonica (GII)*, treated with *Calcareaphosphorica (GIII)*, treated with *Calcarea Fluorica (GIV)* and treated with the combination of *Calcareacarbonica, Calcareaphosphorica and CalcareaFluorica (GV)*. (*) Statistically significant difference compared to group (GI), p≤0.05.

Mean values and standard deviation of sodium concentration (mEq/mg tissue) in dental pulp of rats from control (GI) and experimental groups (GII, GIII, GIV, GV) are shown in figure 12 and table 1. No statistically significant differences in sodium concentration were observed when comparing the groups evaluated in this study. Graph 4 - Sodium concentration (mEq/mg tissue) in dental pulp of rats in the groups: control (GI), treated with *Calcareacarbonica (GII)*, treated with *Calcareaphosphorica (GIII)*, treated with *CalcareaFluorica (GIV)* and treated with a combination of *Calcareacarbonica, Calcareaphosphorica and CalcareaFluorica (GV)*. Table 1 - Concentration of calcium, phosphorus, potassium and sodium (mEq/mg tissue) in dental pulp of rats in the groups: control (GI), treated with *Calcareacarbonica (GII)*, treated with *Calcareaphosphorica (GIII)*, treated with *CalcareaFluorica (GIV)* and treated with the combination of *Calcareacarbonica, Calcareaphosphorica and Calcarea Fluorica (GV)*. Statistically significant differences are represented by different letters comparing the groups in each parameter studied by Analysis of Variance and Tukey's Post-test, p≤0.05.

# DISCUSSION

The choice of Calcareacarbonica 6CH, Calcareaphosphorica 6CH and Calcareafluorica 6CH was also due to the results achieved in previous experiments. [1, 2, 3] Because they are free of side effects and are able to help balance the systemic absorption of Ca, P, Mg, Cl, K, and Na, present in the human body or ingested as food, they also influenced the choice of medication [11, 16, 17]. The traumatic stimulus required to cause the changes in Ca, P, K and Na levels and calcification was provided by the experimental animal model in this paper. Continuously growing incisor teeth, characteristic of rodents, enable assays to investigate possible changes in Calcium levels [18,19,20]. Dental pulp as the target tissue of this investigation added relevance as it is formed of vascularized connective tissue, rich in odontoblasts responsible for dentin production and odontoblasts responsible for dentin demineralization[4]. Dentin-pulp complex exhibits exquisite regenerative potential in response to injury and traumatic stimulus[19,20]. The role of odontoblasts in the transport of $Ca^{2+}$ions and inorganic phosphate (Pi) during dentin mineralization was previously demonstrated[21].The increased (Ca), (P), (K) pulpal indices of this study complement the literature record in that the specific mechanism of transmembrane ionic calcium transport promotes the increase of its concentration over the layer of odontoblasts towards the mineralization line[4, 5, 18, 19, 21]. Drug administration time, 21 days in a daily dose of 03 drops orally, was adequate for stimulation and increases in pulpal inorganic substance levels, as it was in other studies of bone stimulation [1, 2, 3,] and metabolism, or body weight gain[22, 23, 24, 25.] Lower dilutions play a valuable role in homeopathic prescribing by promoting organotropic effects[22,26]. Homeopathic medicines in low potencies have also demonstrated efficacy in clinical trials[1,2,3,4,22,25].

The relation of statistically significant increases in calcium, phosphorus and potassium levels compared to the absence of changes in sodium levels in the pulp tissue observed in this experiment is coherent with the participation of sodium in the maintenance of hydroelectrolytic balance, in the transmission of nervous impulses and in muscle contraction. Intracellular calcium transport is dependent on Na+K+-ATPase located in the basolateral membrane (sodium-potassium pump), where calcium and sodium influx and efflux mechanisms regulate intracellular calcium concentration. In the absence of these mechanisms, the cells would not maintain the calcium concentration and there would be accumulation of the ion in the intracellular environment[27]. The findings in dental pulp of Wistar rats confirmed the ability of the homeopathic medicines *Calcareacarbonica 6CH*, *Calcareaphosphorica 6CH, Calcareafluorica 6CH* to change the levels of inorganic substances: Ca, P; K and Na. *Calcareacarbonica 6CH* has the ability to promote calcification and mineralization events due to the presence of calcium carbonate. According to records in homeopathic pharmacies, the action of these drugs occurs in the interstitial exchanges of tissues, in the nutrition of leukocytes and in the development of bones and marrow. [28, 29, 30]. The presence of *Calcareacarbonica* in groups G(II) and G(V) showed that the concentration of calcium in the pulp tissue, which was so expressive and significant in these groups, is due to its elective action on the bone and articular system[16, 28, 29, 30, 31, 32]. The highest rate of pulp phosphorus found among the groups in this experiment was in the treatment with the combination of *Calcareacarbonica 6CH, Calcareaphosphorica 6CH and Calcareafluorica*. The interaction between the homeopathic calcareas showed superiority in relation to the action of the[16, 28, 29, 30, 31, 32]. The results of group G (IV), regarding potassium levels in the pulp tissue, are justified by the properties of *Calcareafluorica* that act on the circulatory system, strengthening small blood vessels and favoring peripheral circulation [16,32]. This is due to the fact that increased potassium intake is potentially beneficial for most people, in the prevention and control of blood pressure, lower rates of stroke, and may also reduce the risk of coronary heart disease and total cardiovascular disease (CVD)[33]. Structures similar to the dental pulp, which is formed by vascularized connective tissue, have regenerative potential and need a powerful and efficient circulatory system[4,5, 6].

Given the results observed here and in agreement with the proposed literature, homeopathic medicines tested in this study have indications in the dental clinic, in cases of pulp therapy, such as in dental traumas and their consequences on mineralized, ligamentous and vascularized tissues of the teeth. Their indications are also understood as preventive, in cases of demineralization of dental structures and in promoting bone remodeling of structures adjacent to the tooth [11, 16, 28, 29, 30, 31, 32]. Findings regarding the capacity of homeopathic medicines as a therapeutic possibility able to help balance the systemic absorption of Ca, P, Mg, Cl, K and Na, present in the human body and associated with the results of this experiment, which satisfactorily answered the questions raised regarding the levels of inorganic compounds in the rat pulp tissues, are an incentive for further

research regarding the potential of action of the substances tested here in other calcium-dependent organs that make up the musculoskeletal, vascular and ganglionic systems[11,17].

## CONCLUSION

Homeopathic medicines *Calcareacarbonica 6CH, Calcareaphosphorica 6CH, Calcareafluorica 6CH* change the levels of inorganic substances: Ca, P; K and Na in the dental pulp of Wistar rats. *Calcareacarbonica* increased the concentration of calcium and phosphate in the dental pulp of rats. *Calcareaphosphorica* showed no effects on the dental pulp when administered individually. *Calcareafluorica* increased the potassium concentration in the dental pulp of rats. The combination of Calcareas increased the concentration of calcium, phosphorus and potassium in the dental pulp of rats.

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