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Evaluation of Physical Properties of Moringa Oleifera-Based Root Canal Sealer- An In Vitro Comparative Study

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ABSTRACT

Objective: To evaluate physical properties of a mixture made by using *Moringa Oleifera* extract and compare it with Endomethasone N for its use as an endodontic sealer.

Methodology: A total of 60 samples of (Moringa Oleifera mixture) ME mixture and Endomethasone N were used and divided into six groups in order to investigate flow, working time, setting time, pH and solubility. These physical properties were tested according to International Standards Organization 6876/2001. T-test was applied and p-value was set at 0.05 in this in vitro analysis.

Results: The ME mixture displayed higher pH values for fresh (9.56 ± 0.04) and set (8.87 ± 0.10) samples at all time intervals ($p < 0.05$). It also showed better flow (21.15 ± 1.21mm) and solubility (0.73 % ± 0.5) when compared with Endomethasone N.

Conclusion: The results indicated better physical properties of ME mixture when compared with Endomethasone N so, ME mixture can serve as a better alternative to other endodontic sealers.

Keywords: Endodontics, Moringa oleifera, Physical properties, Root canal sealer.

Authors' Contribution:
[1,2]Conception; Literature research; manuscript design and drafting; [3,4]Critical analysis and manuscript review; [5,6]Data analysis; Manuscript Editing.

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Introduction

Root canal treatment (RCT) is considered as the most common and an important procedure in dentistry, usually indicated in cases of irreversible pulpitis. It involves a series of steps which involves removing the contents of root canal followed by replacing it with a core material which is coated with a sealer.[1] The purpose of root canal sealer is to create a three-dimensional hermetic seal and stop entry of microbes from the oral cavity in all directions.[2] Endodontic treatment can fail due to many reasons however mainly the failure occurs if the root canal is not sealed properly. Other causes of failure are; procedural errors and persistent microbial infections. In order to assure good prognosis of endodontic treatment, choice of good endodontic sealer is necessary.[3] Root canal sealers can be classified in various ways depending upon factors such as setting reaction and composition. These include resin based, eugenol, glass ionomer sealers and calcium hydroxide. The basic function of an endodontic sealer is to provide lubrication in the

canal space as well as fill in the voids and irregularities by gliding in the canal and provide a good seal with gutta percha points.

Therefore, the success of an endodontic treatment is also directly linked with the physical properties of the sealer i.e., it needs to have good flow. As the sealer comes in contact with the periapical tissue, it must be biocompatible and has minimal cytotoxic effects.[3,4]

In the last decade, great emphasis has been given to find alternative treatment modalities to fight various infections as the overuse of antibiotics has led to emergence of various multi drug resistant strains of bacteria. One of the substitutes is medicinal plants, being widely used for the treatment of infections and offers a natural mode of treatment. Many studies done in the past have proven the efficacy of drugs derived from herbal extracts used for oral diseases.[5,6] However, there is paucity of evidence with regards to their use against oral infections.

Moringa oleifera has been widely used as a source of food as well as in treatment of various diseases. Many studies done in the past have suggested the nutritional and anti-microbial efficacy of this plant as the biologically active components are investigated.[7] The therapeutic potential and medicinal properties of the plant are extensively reviewed. Various studies have been done on the physiological and pharmacological activities of the leaves, seeds, bark, roots, sap, and flowers of *M. oleifera.*

In one study using Moringa oleifera extract to form a ME mixture, which was used as a root canal sealer by the author, the antimicrobial efficacy of ME mixture came better in comparison with Endomethasone N.[10]

This study aims to conduct evaluation of physical properties of *Moringa* extract by synthesizing a mixture with it that can be used as a potential sealer in future.[8]

Following this, the mixture of *Moringa* extract was compared for its physical properties to a standard Endomethasone N.

Methodology

This In Vitro Comparative study was conducted at Islamic International Dental College from February 2020 to March 2021. *Moringa oleifera* plants were obtained from Nursery farms (Islamabad). They were identified at Herbarium of Pakistan, Quaid-e-Azam University, Islamabad. The ME mixture was synthesized according to the authors' composition at the Riphah Institute of Pharmaceutical Sciences, Islamabad.

The five chemicals which were used for the study include Zinc oxide, Thymol iodide, Eugenol, Magnesium stearate from Daejung (Korea) ®, Barium sulphate from VWR chemicals ® and Hydrocortisone acetate from AK scientific.[8]

In order to conduct this study, two groups of samples were made: *M.oleifera* extract mixture and Endomethasone N (Spécialités Septodont, Saint-Maur-des-Fossés, Cedex, France). Each group comprised of 30 samples. Thus, a total sample of 60 sealers was used for the purpose of this study.

The extract was taken from the plant leaves and it was lyophilized to extend its shelf life. In order to make ME mixture, basic sealer composition was used; Zinc oxide, Barium sulfate, Hydrocortisone acetate, thymol iodide and Magnesium stearate powder. Afterwards, MO extract with various concentrations were incorporated to basic sealer composition and thoroughly mixed. At the end, eugenol was introduced and it was mixed until a homogenous mixture was obtained.[8]

The physical properties were investigated according to ISO 6876/2001 specifications. These include pH, working time, solubility setting time, and flow.[9]

For testing flow, a disposable 03ml graduated syringe was used. The sealer was taken in a volume of 0.05 ± 0.005ml which was later placed on a centre of glass plate with dimensions of 40 x 40 x 5mm^3. Another glass plate measuring 20g and 100g weight were placed on the top after three minutes. After 10 minutes from the start of mixing, the load was removed. A digital caliper (Cole-Parmer Canada.,

Montreal, Canada) was used to measure the sample's disk diameter. The diameter (maximum and minimum) was measured with a resolution of 0.01mm. This procedure was done five times for both sealers; mean calculated in millimeters, was considered.

In order to check the working time, a volume of 0.05 ± 0.005ml sealer was put on the centre of a glass plate with dimensions of 40 x 40 x 5mm.[3] Two glass plates measuring 20g and 100g weight were placed on the top after three minutes. After 10 minutes from the start of mixing, the load was removed. The working time is considered when manipulation of the sealer will not have any side effects on its physiochemical characteristics. The experiment was repeated five times with newly mixed sealer until its diameter was decreased 10% in comparison to the flow test.

For the purpose of this procedure, stainless steel molds with a mm height and 10mm diameter were used. A glass mold was placed underneath the molds before packing them with sealers. The glass mold was transferred to an incubator with 37°C and greater than 95% relative humidity. A Gilmore needle with an active tip of 02mm diameter and weight of 100g was used to determine the setting time.

Later on, the indenter needle was lowered in a vertical position on the horizontal surface of the sealer. The point when the needle failed to make an indentation on the surface was considered as the setting time. Before each test, the tip of the needle was cleaned. Both sealers were tested and five measurements were taken for each sealer. The setting time was stated as time from the start of mixing until the sealer will be set. Three measurements were made for sealer with ME mixture and Endomethasone N.

The solubility of the sealers was defined as "percentage of the mass of specimen material removed from the distilled water compared with the original mass of the specimens". For the purpose of assessing the solubility of sealers, the procedure required stainless steel rings for each sealer having an inner diameter of 20mm and 1.5mm thickness. A glass mold was taken and it was covered with a sheet of cellophane, placed underneath the molds before packing them with sealers. The filled molds were placed in an incubator at 37^{o}C and greater than 95% relative humidity for duration of 50% longer than the setting time. The sealers were weighed three times after their removal from the molds. A petri dish was pre-weighed and 50ml distilled water was poured in it. Two samples were put in a petri dish and were incubated at 37^{o}C and greater than 95% relative humidity for 24 hours. A total of 2–3ml distilled water was used for rinsing the samples, and the washings were drained back into the petri dish. The petri dishes were dried at 110°C in an oven and were reweighed after being cooled in the desiccator to room temperature. The difference between the initial and the final mass of the petri dish was measured and was denoted as the amount of sealer removed.

Fresh and set samples were made for the purpose of this procedure. Fresh samples were labeled as samples mixed immediately after manipulation and the samples mixed and later kept in an incubator for three times the measured setting time (Endomethasone N) at 37°C and greater than 95% relative humidity were marked as set samples. Afterwards, five samples were made for both groups. For making sample discs, rubber molds with 1mm thickness and 5mm diameter were used. A polyethylene tube containing 10ml distilled water was used to place and store the set samples (0.02ml) at 37°C during the study period. A volume of 0.02ml of fresh sample was placed in a flat-bottom container with 10ml distilled water at 37^{o}C. The pH of the solution was measured by using a previously calibrated digital pH meter (SB70P VWR Symphony; VWR International LLC, Radnor, PA) after a predetermined time (0, 20, 60, 80 and 120 minutes for fresh and set samples). The data were entered and analyzed using SPSS version 25.0. Mean and the standard deviation were described for flow,

solubility, setting, and working time for ME mixture and Endomethasone N. In order to compare the pH of ME mixture and Endomethasone N, mean pH values of ME mixture and Endomethasone N were compared and analyzed using independent sample T-test. A value of 0.05 was considered to be significant for analysis of all variables.

The Ethical approval was taken from ethical committee of Islamic International Dental College (Ref. No. IIDC/IRC/2020/001/004) dated 07-01-2020.

Results

The results of flow test indicated that both sealers showed a flow greater than 20 mm, which are in accordance with the ISO 6876/2001 recommendations. The (Moringa Oleifera: ME) ME mixture had a higher flow in comparison with Endomethasone N. The ME mixture showed 21.15 ± 1.21 mm whereas Endomethasone N value was 18.02 ± 0.6 mm.

The results showed that working and setting time of Endomethasone N was according to the values mentioned by the manufacturers, *Septodont*. The working time of Endomethasone N was 11.0 ± 0.5 hours and setting time was 4.5 ± 1.3 hours. The ME extract had comparable working time (11.1 ± 0.8 hours) but setting time was longer (5.31 ± 0.98 hours). Both sealers demonstrated solubilities within the range (3% mass fraction) allowed in the ISO 6876/2001 recommendations but, Endomethasone N showed highest value of solubility (0.78% ± 0.2). Although the fresh and set samples of both sealers (Endomethasone N and ME mixture) produced an alkaline pH, the ME mixture showed a higher pH value as shown in Table II. The results were expressed as mean ± standard deviation (n=5).

The p-values for fresh and set samples were found to be statistically significant (<0.05) than the Endomethasone N at all time intervals.

Table I: Physical properties of sealers

Properties	Endomethasone N	ME mixture
Flow (mm)	18.02 ± 0.6	21.15 ± 1.21
Working time (hours)	11 ± 0.5	11.1 ± 0.8
Setting time (hours)	4.5 ± 1.3	5.31 ± 0.98
Solubility (%)	0.78 ± 0.2	0.73 ± 0.5

Table II: Comparison of pH values of Endomethasone N and ME mixture, expressed as mean ± Standard deviation for fresh sample alongwith their p-values.

Time (mins)	**pH of Endomethasone N (Fresh Sample)**	**pH of ME mixture (Fresh Sample)**	**P value**
0	7.1 ± 0.00	8.25 ± 0.04	0.000001*
20	7.31± 0.10	8.38 ± 0.07	0.0001*
60	7.38 ± 0.05	8.43 ± 0.08	0.00004*
80	7.47 ± 0.01	8.6 ± 0.05	0.000003*
120	7.74 ± 0.03	9.56 ± 0.04	0.0000006*

* = Significant at p value <0.05

Table III: Comparison of pH values of Endomethasone N and ME mixture, expressed as mean ± Standard deviation for Set sample alongwith their p-values.

Time (mins)	**pH of Endomethasone N (Set sample)**	**pH of ME mixture (Set sample)**	**P value**
0	7.58 ± 0.07	8.26 ± 0.06	0.0002*
20	8.09 ± 0.06	8.4 ± 0.06	0.0049*
60	8.23 ± 0.09	8.48 ± 0.05	0.0157*
80	8.33 ± 0.11	8.64 ± 0.03	0.0125*
12	8.48 ± 0.07	8.87 ± 0.10	0.0054*

* = Significant at p value <0.0

Discussion

Endodontic treatment is one of the most common procedures in clinical dentistry. With newer root canal sealers being developed, clinicians need to know and understand physiochemical characteristics of the product being used for

obturation.[10] The use of herbs for the prevention and cure of different diseases has been in use since centuries across various countries.[11] Many researchers now believe that the use of different herbal extracts and their phytochemicals in traditional herbal medicine is a good alternative to synthetic chemical agents.[12] The characteristics of root canal sealer are mainly depending on the kind and amount of chemical constituents. Therefore, the aim of study was to assess the physiochemical characteristics of ME mixture and compare this with another established sealer Endomethasone N. The significance of exploring these parameters justifies the safe use of newer endodontic sealers.

According to ISO 6876/2001, on spread/flow, an optimal endodontic sealer should have a diameter of at least 20mm.[12] Our findings indicated that ME mixture had a mean diameter of 21.15 ± 1.21 mm, thus, conforming to the recommendation. Endomethasone N, on the other hand had a diameter of 18.02 ± 0.6 mm, which is less than the recommended diameter. The results for spread of Endomethasone N were in agreement with a study which reported a spread diameter of 18.76 mm.[13] The results for flow of ME mixture were similar to a study done by Torres, to evaluate the flow and filling of root canal sealers using different methodologies. The spread diameter of AH plus sealer flow was found to be 21.39 ± 0.76 mm.[14] The results for flow of ME mixture were comparable to the flow of Radic-Sealer (20.80 ± 0.84 mm) in a study which evaluated the physiochemical properties of bioceramic and epoxy resin based root canal sealers.[15] Several factors may affect the penetration of endodontic sealers within constricted regions of the root canals which includes obturation technique, contact area and the sealer's flow rate.[16] The root canal system has narrow irregularities in dentin, secondary and accessory canals, voids between master and accessory cones, all of which necessitates suitable flow properties in root canal sealer. If there is extreme reduction in flow and working time, it results in an inability to work effectively with a material, increasing the chances of a void being created. On the other hand, the sealer should flow into accessory canals and between gutta-percha cones, without increasing the risk of periapical extrusion.[17]

The setting and working time of Endomethasone N was in accordance to the manufacturer's recommendation, Septodont. The working time of ME mixture was also comparable to Endomethasone N. The lack of relevant literature regarding regarding setting time makes it difficult to corroborate the result from previous studies.The change in setting time may be due to room temperature, relative humidity and particle size.[18] Setting time influences clinical working, too fast or slow setting time will affect the prognosis of treatment. If the setting time is slow, it will allow sealer to be placed in more than one canal as well as the ability to recover gutta-percha from the canals directly after obturation, if necessary. Coronal leakage may take place shortly after the completion of root canal treatment if the setting time is slow. If the sealers are unset or partially set, they may allow rapid diffusion of bacteria or bacterial byproducts.[19]

One of the most desirable physical properties for root canal sealers is insolubility as it has great importance in the success of endodontic therapy.Low solubility is a requirement (No.57) as mentioned in the ANSI/ADA specification 2000 and in International Standards Organization 6876/2001 standard for root canal sealing materials.[21]

The solubility of the set material should not exceed 3% mass fraction after immersion in water for 24 hours when tested according to International Standard 6876/2001.[21] The results of study showed that both materials were within the range recommended by ISO standard. Endomethasone N was most soluble with mean solubility of 0.78%, while ME mixture had a mean solubility value of 0.73%. The results for Endomethasone N were in corroboration with a study done by Poggio et al where the highest solubility values were found in samples obtained with Zinc oxide eugenol-based

cements (Argoseal – 0.77% and Endomethasone C – 0.73%). The zinc oxide eugenol-based sealers exhibit almost 1% - 7% weight loss after being stored in water.[22]

The physiochemical properties of both sealers were tested according to the guidelines mentioned in ISO 6876/2001.[12] Among the materials tested ME mixture showed highest alkaline pH value for fresh (9.56 ± 0.05 at 120 minutes) and set (8.87 ± 0.10 at 120 minutes) samples. This may be because of Magnesium (Mg), Potassium (K) and Calcium (Ca) present in abundance in the M.*oleifera* tissues. The vegetative parts and immature pods have the highest potassium content, seeds and leaves contain calcium and Magnesium in abundance.[23] The pH results of our study for fresh samples of Endomethasone N ranged from 7.1 to 7.74. These results were in agreement with those found by Pawińska et al. where the pH for Endomethasone N ranged from 7.09 to 7.71. The same study reported higher pH results for the ME mixture (8.25 – 9.96) in comparison to Gutta flow (5.02 – 6.60), Gutta percha (6.05 – 6.70), Resilon (5.70 – 7.45) and Tubiseal (6.85 – 7.83).[24] The range of our findings for the fresh ME mixture (8.25-9.96) were similar to the previous findings by Zhou et al. while evaluating MTA- F, Thermaseal and AH plus.[12] This was matched by bar chart values. The authors failed to report the exact pH values from their research. The success of RCT depends on the maintenance of alkaline environment during the procedure and after its completion.[25] The reduction of bacterial load effects the periodontal inflammation and healing of periapical tissues. Alkaline pH provides a favorable environment for the sealer's antibacterial activity and healing. The alkaline pH creates optimum conditions for working of alkaline phosphatase which helps in dephosphorylating compounds to work on periodontal tissues and stimulates bone remineralization.[26,27] On the contrary, the endodontic sealers with low pH values increase the osteoclastic activity and facilitate bone resorption.[28]

Due to limitations of resources, a formulation was not made by using *Moringa* extract. The benefit of making a formulation is that quantity of active component is uniform. It is easy to handle and administer and has better shelf life. Various formulations can be made by using different ratios of *Moringa* extract and characterized by applying various tests such as content uniformity test.

Candida albicans is most commonly found fungi in the oral cavity but in our study the antifungal properties of the ME mixture were not evaluated. It is recommended to investigate variables such as antiallergic, cytotoxicity and antifungal properties of formulation to further ensure its safe usage. A direct contact test or collecting the flora from the root canal can also be performed to check the antimicrobial efficacy. Variables such as viscosity, film thickness and dimensional stability were not tested for ME mixture due to lack of viscometer and other equipment.

Conclusion

The emergence of natural compounds in medicines ensure good biocompatibility and less side effects. Moringa Oleifera offers a good, natural, readily available and cost-effective alternative. This study highlighted the physical properties of *Moringa* extract as a potential sealer. This ME mixture displayed higher pH values for fresh and set samples at all time intervals, better flow (21.15 ± 1.21mm) and solubility (0.73 % ± 0.5) when compared with Endomethasone N. The results for ME mixture were better than Endomethasone N in terms of antimicrobial efficacy and physical characteristics. In view of these results, ME mixture can serve as a good alternative for other unnatural endodontic sealers.

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