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Editorial

PERCEPTIONS! PERCEPTIONS!

Many research works begin with perceptions. What is perception?

Perception is the organization, identification, and interpretation of information gathered by the sensory system, “to represent and understand the presented information or environment.”

Longman Dictionary of Contemporary English, Pearson Education Limited, 2003, defines perception as: The way you think about something and your idea of what it is like; the way that you notice things with your senses of sight, hearing, etc.; and the natural ability to understand or notice things quickly.

Living in today’s environment and its conditions (circa 2021, coronavirus pandemic) heightens one’s perceptive abilities or dulls it altogether. Why do I say that? This pandemic is a two-edged sword that has altered our life, our lifestyle, our way of thinking/perceiving and doing things. But as nature wills it, man adapts, and fortunately, Darwin’s word proves that the fit survives. This fitness includes all aspects of existence, physical, mental, emotional, spiritual, and virtual. This fitness requires that all systems are on “go” mode, no matter what. This fitness allows people to see and recognize patterns, utilize this in facing and seeking solutions to problems. This fitness spells the difference between surviving, thriving and stagnating, losing.

Who thrives? Those who seize the opportunity to gain knowledge from happenings, those who study and respect learning, those who follow their perceptions which lead them to grow and flourish.

Soledad V. Navarro

Effectiveness of Gentian Violet in a Pencil Core as Marking Tool Used on Human Oral Mucosa

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ABSTRACT

Introduction: Indelible pencils are commonly used in Dentistry to outline the posterior vibrating line to ensure the posterior palatal seal and to demarcate incision lines. Popular indelible pencils contain an aniline dye, primarily the Gentian Violet (GV). However, aniline dyebased markers were found to contain either a harmful or toxic concentration of GV, which resulted in restrictions on the use of GV. For this reason, this study formulated an indelible pencil, with a non-harmful concentration of GV, and evaluated its potential to mark the oral mucosa, and to transfer the mark to an alginate impression. ***Methodology***: This exploratory study consisted of 2 phases: the identification of a non-toxic GV amount in a pencil core and the evaluation of its marking ability and color transferability. Using a 5- Likert scale, each participating clinician rated the marking quality as to coloring ability, coloring continuity, color intensity and definiteness of the mark; and color transferability as to coloring continuity, color intensity and definiteness of the mark. ***Results***: The amount of GV in a 1.5-inch stroke was 0.00013g, which is way below the toxic dose of 0.001g in a 50kg individual. Results of the marking ability of GV showed excellent rating, which was statistically significant across the ratings in all the criteria. However, the rating on transferability of GV unto the alginate impression was not statistically significant across the ratings in all criteria. ***Conclusion:*** The nontoxic amount of GV for every 1.5-inch stroke was identified in this study. As a marking tool, the GV pencil core was found to be effective in marking the human oral mucosa, but less effective when transferring the mark to an alginate impression. This area should be further studied to have a more relevant meaning to the purpose of using an indelible pencil in Dentistry.

INTRODUCTION

One of the indispensable tools in Dentistry is the indelible pencil, used to outline anatomical landmarks on the oral mucosa. Also known as copying pencil, indelible pencils are made of graphite, clay, and dye. In the 19th century, the most common dye used then was the aniline dye methyl violet[3], which further developed into multiple derivatives, paving way for gentian violet as the most commonly used dye up to this day.

Gentian Violet (GV), also known as crystal violet, is a water-soluble, basic cationic dye that forms affinity with a surface that has an opposite charge[1]. Through this ion interaction, GV is adsorbed onto the surface to impart its color. However, the color quality of the imprint made by a dye depends on its concentration. According to Mitchell[8], copy-

-ing pencils contain <25-50% dye. Unfortunately, this is the only available data pertaining to the approximate amount of dye in copying pencils because its history is not well documented, and proportions of its components differ among brands, depending on the manufacturer.

However, GV is also employed in microbial staining as the primary dye to a heat-fixed smear for gram staining. As a staining material for microbial cells, the positively-charged crystal violet permeates through the bacterial cell wall and membrane[11], and links with the negatively-charged units of the bacterial cell via ion exchange[7]. The concentration of GV as a primary dye in gram staining is from 0.3% - 2%, depending on the duration of decolorization time with alcohol[13].

Further, GV also has an extensive history as an anti-bacterial and anti-fungal agent. It is popularly used to manage oral candidiasis at 1% concentration. Its usefulness expanded through the years as new anti-parasitic and anti-angiogenic therapies[6]. However, there were reports about the potentially harmful and toxic effects of GV when used as medicinal agents. According to Docampo and Moreno[2], oral administration of GV can cause gastrointestinal irritation and can potentially decrease white blood cell count when introduced intravenously; but no side effects were reported when topically applied. Subsequent animal studies also reported the potential association of GV with the development of cancer, which led to some restrictions on the use of GV.

In 2019, OEHHA[12] issued the full report on the carcinogenicity of GV, including it in "Proposition 65 List of chemicals known to cause cancer". These controversies about GV affected the availability of indelible pencils for use in Dentistry, which is employed as a marking tool in various dental procedures. Gentian violet has been the dye used as an indelible pencil colorant because of its brilliant violet hue, high tinge value, and longevity[3]. These qualities of GV are useful when outlining the mesial surfaces of both abutment teeth aiming to be cut parallel with each other for the preparation of an anterior fixed bridge[9]; marking the posterior vibrating line in the fabrication of a complete denture5;, and as a surgical marker in procedures such as lip repositioning and reduction of gingival display methods[10]. But considering the amount of GV that may be associated with these procedures, the researchers deem that only a small amount of GV is utilized, which translates to a lower probability of ingesting a harmful or toxic GV dose. According to Gosselin, Hodge, Smith and Gleason[4], the reported probable oral lethal dose of GV for humans is 0.05g-0.5g/kg-body weight. Considering the GV concentration range to color a living cell, which is 0.3% - 2%, the anti-fungal concentration of 1%, and the oral

lethal dose of 0.05g-0.5g/kg-body weight, this study explored the minimum nonharmful or nontoxic concentration of GV in a pencil core that can effectively color the oral mucosa, and concomitantly transfer the color to an impression. This product will provide benefit to dental clinicians when outlining the posterior vibrating line confirming the posterior palatal seal for complete dentures, and when demarcating incision lines in surgery without causing any adverse effects.

METHODOLOGY

This exploratory-descriptive research design consisted of 2 phases: the identification of the minimum non-harmful or nontoxic concentration of GV in a pencil core that leaves a mark on silicone rubber material and the evaluation of its marking ability on the oral mucosa and color transferability on alginate impression. Different amounts of GV (0.1g, 0.2g, 0.4g, 0.6g, 0.8g, and 1g) were combined with corresponding amounts of stearic acid, beeswax, and coconut oil, which are the basic materials used in a wax-based crayon to yield a total volume of 5ml (Table 1). The mixture was cooled then poured into a drinking straw to produce a cylindrical pencil core. The resultant length of the solidified mixture was 5 inches (Figure 1). The pencil cores were tested on moist silicone rubber cheeks by making a 1.5-inch stroke with moderate pressure, and evaluated for marking ability as to coloring, continuity, intensity, and definiteness of the mark by the researchers, using a 5-Likert Scale (Table 2). The identified amount of GV that effectively left a colored mark was 0.4g/5ml. To measure the GV amount used to make a 1.5-inch mark, half-inch of the pencil core was spent to make strokes on bond paper (Figure 2). The remaining length of the pencil core was subjected to a water displacement test to determine the average weight of GV per 300 strokes. A graduated cylinder was filled with 20ml of water and the remaining pencil core was submerged. The resulting water volume was measured. After computing for the eventual amount of GV in a 1.5-inch stroke, the study underwent ethics review by the Ethical Review Committee (ERC) of UE-Office of Research Coordination, in preparation for the second phase.

Twenty edentulous patients and 40 clinicians were recruited for the study, using the Informed Consent and Data Privacy statements approved by the ERC. Each patient was evaluated by 2 clinicians. Using the pencil core with 0.4g/5ml of GV, a stroke was made on the posterior vibrating line of the edentulous patients and rated for its marking ability by the participating clinicians, using the same criteria for evaluation as used in the silicone rubber cheek.

After rating its marking ability, an impression was taken using alginate impression material for the rating of color transferability as to color continuity, intensity, and definiteness of the mark (Table 3) by the same participating clinicians.

Table 1. *Composition of pencil core with different amounts of gentian violet*

MATERIALS	CONCEN-TRATION #1	CONCEN-TRATION #2	CONCEN-TRATION #3	CONCEN-TRATION #4	CONCEN-TRATION #5	CONCEN-TRATION #6
Gentian Violet	0.1g	0.2g	0.4g	0.6g	0.8g	1g
Stearic Acid	3g	2.9g	2.8g	2.7g	2.6g	2.5g
Beeswax	1g	0.9g	0.8g	0.7g	0.6g	0.5g
Coconut oil	1ml	1ml	1ml	1ml	1ml	1ml

Table 2. *Marking ability criteria and 5-Likert Scale*

CRITERIA	EXCELLENT (5)	GOOD (4)	FAIR (3)	POOR (2)	VERY POOR (1)
COLORING ABILITY	Evident mark	Slightly evident mark	Fair mark	Inadequate mark	No mark
COLOR CONTINUITY	Consistent solid line without interruption	With one to three interruptions	With four to six interruptions	With seven to nine interruptions	With ten or more interruptions
COLOR INTENSITY	Very Strong	Strong	Strong	Weak	No color
DEFINITENESS OF THE MARK	Highly defined boundaries	Well defined boundaries	Defined Boundaries	Slightly defined boundaries	Ill-defined Boundaries

Figure 1. *Solidified pencil core*

Figure 2. *Number of 1.5-inch strokes that half-inch of pencil core makes*

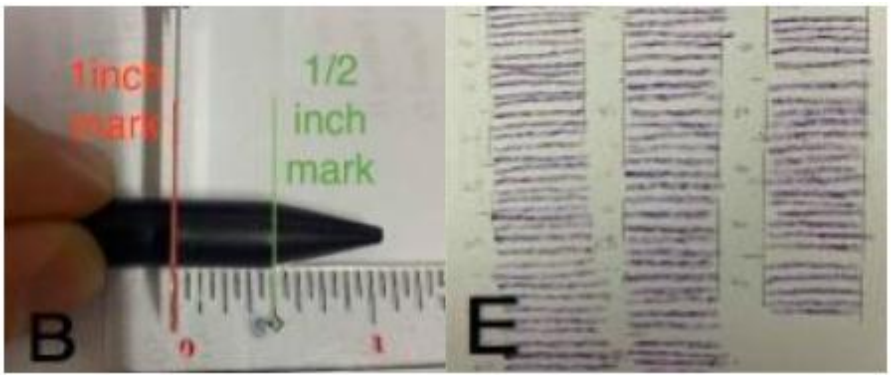

Table 3. *Color transferability criteria and 5-Likert Scale*

CRITERIA	EXCELLENT (5)	GOOD (4)	FAIR (3)	POOR (2)	VERY POOR (1)
COLOR CONTINUITY	Consistent solid line without interruption	With one to three interruptions	With four to six interruptions	With seven to nine interruptions	With ten or more interruptions
COLOR INTENSITY	Very Strong	Strong	Strong	Weak	No color
DEFINITENESS OF THE MARK	Highly defined boundaries	Well defined boundaries	Defined Boundaries	Slightly defined boundaries	Ill-defined Boundaries

RESULTS AND ANALYSIS

The first phase aimed to determine the minimum non-harmful concentration of GV that can effectively leave a colored mark on the silicone rubber cheek. Results showed that the number of 1.5-inch strokes that half-inch of the pencil core would make was on average, 300 strokes. The water displacement test resulted in a loss of 0.5 ml after subtracting the original volume (20ml) from the resultant water volume with the pencil core (24.5ml) measuring 4.5 inches. This means that 300 strokes of 1.5-inch length lose 0.5ml. Computing for the actual amount of GV used in half-inch pencil core to make 300 strokes, the 0.4g GV in 5-inch pencil core was multiplied by 0.5ml volume loss, and the product was divided by the 5ml total volume of the pencil core before solidification. This algebraic equation yielded to 0.04g/1.5-inch length of the pencil core. The quotient of 0.04g was then divided by the 300 strokes to get the approximate amount of GV per stroke, which was 0.00013g/1.5-inch stroke.

The second phase aimed to evaluate the marking ability and color transferability of the pencil core. Results showed that clinicians rated the marking ability of the pencil core as excellent in all the criteria (Table 4). Coloring ability was rated excellent by 90% (36 out of 40) of the clinicians; while only 10% (4 out of 40 observers) rated good. Similarly, color continuity was also rated by 72.5% (29 out of 40) of the clinicians; while 27.5% (11 out of 40 observers) rated good. In terms of color intensity, 57.5% (23 out of 40) of the clinicians gave an excellent; 25% (10 out of 40) rated good and, 17.5% (7 out of 40) rated fair. Lastly, 57.5% (23 out of 40 observers) rated excellent, 35% (14 out of 40 observers) rated good and 7.5% (3 out of 40 observers) rated fair on definiteness of the mark.

Table 4. *Distribution of marking ability ratings*

CRITERIA	COLORING ABILITY	COLOR CONTINUITY	COLOR INTENSITY	DEFINITENESS OF THE MARK
EXCELLENT	90.0% (36)	72.5% (29)	57.5% (23)	57.5% (23)
GOOD	10.0% (4)	27.5% (11)	25.0% (10)	35.0% (14)
FAIR	0	0	17.5% (7)	7.5% (3)
POOR	0	0	0	0
VERY POOR	0	0	0	0
TOTAL	100.0% (40)	100.0% (40)	100.0% (40)	100.0% (40)

To evaluate the distribution of the proportions of marking ability rating across the different criteria, Z-test at α=.05 was used (Table 5). Analysis of results showed that the proportions of ratings in all criteria were significantly different. The proportions of coloring ability rating were significant, $z= 7.155$, $p<0.001$; color continuity, $z=4.025$, $p<0.001$; color intensity, $z=2.952$, $p= 0.00318$; and definiteness of the mark, $z=2.018$, $p=0.0434$.

Table 5. *Z-test of the proportions in the different criteria for marking ability*

COLORING TEST	COLORING ABILITY	COLOR CONTINUITY	COLOR INTENSITY	DEFINITENESS OF THE MARK
Z	7.155	4.025	2.952	2.018
P	<0.001	<0.001	0.003	0.043

As to color transferability, the ratings were different across the criteria, with good ratings having the highest proportions (Table 6). Coloring continuity was rated excellent by 42.5% (17 out of 40) of the clinicians evaluated excellent; 45% (18 out of 40) as good; 10% (4 out of 40) as fair; and 2.5% (1 out of 40) as poor. Color intensity was rated excellent by 47.5% (19 out of 40) of the clinicians; 30% (12 out of 40) as good; 20% (8 out of 40) as fair; and 2.5% (1 out of 40) as poor.

As to definiteness of the mark, 32.5% (13 out of 40) of the clinicians rated excellent; 40% (16 out of 40) rated good; 20% (8 out of 40) rated fair; and 7.5% (3 out of 40) rated poor.

Table 6. *Distribution of color transferability ratings*

CRITERIA	COLOR CONTINUITY	COLOR INTENSITY	DEFINITENESS OF THE MARK
EXCELLENT	42.5% (17)	47.5% (19)	32.5% (13)
GOOD	45.0% (18)	30.0% (12)	40.0% (16)
FAIR	10.0% (4)	20.0% (8)	20.0% (8)
POOR	2.5% (1)	2.5% (1)	7.5% (3)
VERY POOR	0	0	0
TOTAL	100.0% (40)	100.0% (40)	100.0% (40)

To evaluate the distribution of the proportions of color transferability ratings across the different criteria, Z-test at α=.05 was used (Table 7). Analysis of results, however, showed that the proportions of ratings in all criteria were not significantly different. The proportion distribution of color continuity rating was not significant ($z = -0.2254$, $p=0.818$); color intensity rating was also not significant ($z= 1.6064$, $p= 0.107$); as well as the definiteness of the mark ($z= 0.6977$, $p= 0.484$).

Table 7. *Z-test of the proportions in the different criteria for color transferability*

COLORING TEST	COLOR CONTINUITY	COLOR INTENSITY	DEFINITENESS OF THE MARK
Z	-0.2254	1.6064	0.6977
P	0.818	0.107	0.484

DISCUSSION

Gentian violet has long been used as an indelible marker colorant because of its brilliant violet hue, high tinge value, and longevity[3]. The GV's ability to color a surface or living organisms has been attributed to its being a basic cationic dye with a positive charge that forms affinity with the negative charge of a surface[1]. Through this ion interaction, GV is adsorbed onto the surface to impart its color. Likewise, GV is recognized in the medical field as anti-bacterial, anti-fungal, antiparasitic, and anti-angiogenic[6]. However, when some studies provided evidence regarding its carcinogenicity, the use of GV was restricted[12], which affected the availability of indelible markers for use in Dentistry. Although GV is used at different concentrations for different purposes, it was not very clear as to what amount of GV can make this dye harmful and carcinogenic.

Considering how essential an indelible pencil is in Dentistry, this study aimed to identify a minimum GV amount that can effectively impart its color on the oral mucosa that can likewise be transferred to an alginate material.

The GV amount tested ranging from 0.1g to 1g considered the available information about the amount of dye in copying pencils, which is <25-50% dye[8]; the 0.3-2% GV concentration used in gram staining[13] and the reported 0.05g-0.5g/kg-body weight probable oral lethal dose of GV for humans[4]. With 0.4g of GV in a 5ml mixture which yielded a 5-inch pencil core that effectively left a 1.5-inch colored mark on the silicone rubber cheek, it was necessary to compute the number of strokes that can be made from this pencil core so that the actual GV amount used to make 1 stroke of 1.5-inch length can be assessed. The water displacement test used to approximate the volume of pencil core spent by half-inch to make 300 strokes applied the Archimedes' principle where the "buoyant force on an object equals the weight of the fluid displaced"[14]. Likewise, the algebraic equations utilized led to the identification of GV weight that makes a single 1.5-inch stroke. Comparing the resultant 0.00013g/1.5-inch stroke with the reported lowest lethal dose of 0.05g/kg-body weight, assuming that an average adult patient for complete denture is 50kg, the 0.00013g was way below the 0.001g (0.05/50kg).

According to Mitchell[8], copying pencils contain <25-50% dye. The formulated pencil core consisted only of 0.0325% GV (0.00013g/0.4g). Despite this low percentage, the overall marking quality rating was still rated with excellent marking ability. This is attributed to the bright violet hue and high tinge value[3] of GV and its ability to interact with the surface that it comes in contact with, as a result of ion exchange[7]. However, the rating in color transferability did not indicate the same effectiveness as its marking ability. Although higher proportions of clinicians rated GV as good in this aspect, its difference with the levels of the 5-Likert scale was not significant. According to Abrahart & Stothers[1], one of the properties of GV is water solubility. It can be that during the impression taking, the water component of the alginate reacted with GV ions, which caused the dispersion of GV on the set alginate. This explains why color continuity, intensity, and definiteness of the mark were compromised. Recognizing this as the weakness of the study, it is recommended that a component that reduces the solubility of GV, such as a mordant, can be included in the formulation of a pencil core.

However, the pencil core with 0.00013g GV per 1.5-inch stroke was justified as non-toxic and found to be an effective marking tool for human oral mucosa.

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Locating Mesiobuccal Canal 2 of Maxillary 1st Permanent Molar using Disclosing Solution and Ophthalmic Dye

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ABSTRACT

Introduction: One of the most challenging parts of endodontic treatment that endodontists encounter, is the anatomical complexities of the root canal. The major cause of the failure of the treatment is the missed canal or an extra root. The root canals of the maxillary first molars have been described as the most complicated morphology of all the maxillary teeth. This is attributed to the high incidence of a second mesiobuccal canal 2 (MB2) in their mesiobuccal roots that is highly variable in its location. Specifically, this study aims to measure the effectiveness of using disclosing solution and ophthalmic dye in locating the Mesiobuccal Canal 2 of the Maxillary 1st Permanent Molar. ***Methodology***: The quantitative, quasi-experimental method of research was conducted on 40 respondents from a 6th year level as clinicians who completed their pre-clinical subject in Endodontics were included in this study. Specimens used are marked as A: without any staining solution; B: with disclosing solution and C: with Ophthalmic dye. The three specimens were properly accessed and cleaned thoroughly with sodium hypochlorite. The disclosing solution and ophthalmic dye were applied on canal orifices, using file #10 to identify its location. ***Results and Conclusion:*** Both dyes worked as a staining solution, however, the majority of the respondents had eased in locating the MB2 canal using Ophthalmic Dye as compared to Disclosing Solution and without any staining solution ($p<.05$).

Keywords: Disclosing solution, Ophthalmic Dye, Root canal, Mesiobuccal Canal 2, MB2

INTRODUCTION

Root canal treatment is a frequently performed procedure aimed to address pulpal and periradicular disease. It includes several clinical procedures regardless of the initial diagnosis. The emphasis of each step varies according to whether there is a vital pulp (non-infected) or if the pulp system contains necrotic, infected tissue and if there is periapical pathology (El-Ma'aita, A.M. et. al, 2015).

Endodontics is a branch of dentistry in which chemical and mechanical remedies are used to put limits on pulpal and periapical diseases. This biologically approved technique has helped periapical tissues in the process of healing significantly (Hassani, N., et. al, 2015).

Awareness and understanding of the presence of unusual external and internal root canal morphology contribute to the successful outcome of the root canal treatment. Maxillary molars show considerable anatomic variation and abnormalities concerning the number of roots and root canals. Traditionally, the maxillary molar has been described to have 3 roots with 3 or 4 root canals, with the fourth canal commonly being found in the mesiobuccal root (MB2). An inability to locate the MB2 root canal in the mesiobuccal root of maxillary molars may be a major cause of the failure of root canal treatment. The presence of patent furcal, lateral and accessory canals are the portals of entry and exit between the root canal space and periodontal ligament (Jasrotia, A., and Sharma, N. 2017).

Anatomical complexities impose limitations to the chemico-mechanical preparation of the root canal, leading to areas not touched by the instrument, resulting in unsuccessful cases. Such complexities are of great importance, especially in maxillary molars. These teeth present a great variety in the number of main canals located in primary treatments. Previous clinical studies reported variation in distobuccal and palatal canals of maxillary molars. The presence of the MB2 canal in maxillary first molars is said to range from 50% to 90% of cases. Knowing the morphology of the root canal system, therefore, is extremely important in planning endodontic therapy, as its success relies on the location of all canals that can then be disinfected, shaped, and filled (Coelho, M., et. al, 2018).

The root canals of the maxillary first and second molars are described as having the most intricate morphology of all maxillary teeth. This is attributed to the high prevalence of a second mesiobuccal canal (MB2) in their mesiobuccal roots that is highly variable in its location. Failure to locate MB2 in maxillary molars has been associated with an increased treatment failure rate (Alfouzan., et. al, 2019).

Root canal failure is a common problem in dentistry. The success of endodontic and re-endodontic treatment depends on many factors. These include periodontal disease, root fractures, residual necrotic pulp tissue, presence of peri-radicular infection, broken instruments, mechanical perforations, root canal underfillings, root canal overfillings, missed canals, or unfilled canals. In the study of Iqbar, A., (2016), according to the tooth type, the majority of the endodontic failures were noted in maxillary molars (44.4%), mandibular molars (20%), and maxillary premolars (15.5%). The endodontic treatment performed by the general dental practitioners (GDPs) showed the most failure rate (78.8%). The factors which were most responsible for endodontic failures were underfilled canals (33.3%), unfilled and missed canals (17.7%).

Disclosing solution is an agent used in staining the tooth, it is used to visualize the dental biofilm on the tooth surfaces. It works by changing the color of the dental plaque. There are also desired properties of an acceptable disclosing solution its color intensity, duration, taste, mucosal irritation, diffusability, and antiseptic action (Datta, D., et. al, 2017).

The purpose of this study is to determine the effectiveness of using Disclosing Solution and Ophthalmic Dye in locating the mesiobuccal canal 2 of maxillary 1st permanent molar.

METHODOLOGY

Research Design

The research utilized a Quantitative, Quasi-experimental method of research. Quantitative research involved the use of computational, statistical, and mathematical tools to derive the results. It was conclusive in its purpose as it quantified the problem and showed how prevalent it was by looking for projectable results to a larger population. The Quasi-experimental method of research involved the manipulation of an independent variable without the random assignment of participants to conditions or orders of conditions.

Sample

The samples consisted of 3 acquired specimens of maxillary first molars and were pre-approved by the Endodontic Department. The specimens were prepared by the researchers under the supervision of the clinical instructors of the Endodontic Department to make sure specimens are properly accessed. They were marked as specimen A, without any staining solutions; specimen B, with disclosing solution; and specimen C, with ophthalmic dye.

The exclusion criteria for the tooth are as follows:

1. Fractured Tooth
2. Tooth with large restoration that compromised the integrity of the root canal system
3. Specimen that canals were previously treated

The rotary (high-speed handpiece, round bur, and non-cutting tapering fissured endo bur) were used in the preparation. The first step was the access preparation of the 3 specimens which will be marked as specimens A, B, and C. Specimen A did not use any

staining solution. The tooth was cleaned thoroughly with 5.25% of sodium hypochlorite and then dried to visualize the anatomy of the chamber floor. The endodontic explorer, tactile sense, and visualization were used to locate the mesiobuccal canal 2. Specimen B was also cleaned thoroughly with 5.25% of sodium hypochlorite, rinsed with 100% ethyl alcohol, and then dried to visualize the anatomy of the chamber floor. To locate the mesiobuccal canal 2, the pulp chamber was flooded with the disclosing solution for 3 minutes until the canal appeared pink in color. The excess stain was washed with water and removed by sodium hypochlorite leaving only the canals stained. Specimen C was cleansed thoroughly with 5.25% sodium hypochlorite and then dried to visualize the anatomy of the chamber floor. To locate the mesiobuccal 2 canal (MB2), the pulp chamber was flooded with fluorescein sodium for 3 minutes until the canal appears blue. The excess stain was washed with water and removed by sodium hypochlorite leaving only the canals stained. The MB2 canal was readily located by the uptake of the dye that emitted bright green fluorescence.

Respondents

A total of 40 respondents from 6th-year level clinicians who completed their pre-clinical subject in Endodontics were included in this study. The study excluded those students who declined to be part of the study.

A Likert-scale researcher-made survey was used to rate the ease of locating the MB2 canals of the 3 specimens.

Procedures

The three (3) prepared specimens were placed on the table marked as A, control specimen; B, a specimen with disclosing solution; and C, a specimen with ophthalmic dye. Respondents entered the room, in a line and asked to locate the mesiobuccal canal 2 using access file number 10. Locating the MB 2 was timed for three (3) specimens. The respondents were asked to rate their experience in locating the canals as 4, with ease; 3, minimal difficulty; 2, difficulty; and 1, no.

Survey forms were collected and placed in a well-sealed brown envelope. Once the survey forms were tallied, placed in a new well-sealed brown envelope together with the survey forms. Tallying of the survey forms was done at home and the results were submitted to a certified statistician for statistical treatment.

Data gathering through a survey was done under the supervision of a clinical instructor from the Endodontics Department to eliminate bias.

RESULTS AND ANALYSIS

Table 1.1 *Distribution of the Respondents according to their Age*

	Frequency (n=40)	Percentage
Age (in years)		
21 – 23	18	45.0
24 – 26	9	22.5
27 – 30	5	12.5
31 – 35	5	12.5
36 – 38	3	7.5
Mean ± SD = 25.78 ± 4.90		

Table 1.1 shows that the age of the respondents ranged from 21 to 38 years with a mean age of 25.78 years. The majority of the respondents ranged from 21 to 23 years of age.

Table 1.2 *Distribution of the respondents according to their Gender*

	Frequency (n=40)	Percentage
Sex		
Male	14	35.0
Female	26	65.0

Table 1.2 presents the majority of the respondents were female (65%).

Table 1.3 *Distribution of the respondents according to their Clinic Level*

	Frequency (n=40)	Percentage
Clinic Level		
2	10	25.0
3	14	35.0
4	16	40.0

Table 1.3 presents the majority of the respondents were enrolled in clinic 4 (40%)

Table 2. *Distribution of Respondents According to their Level of Difficulty when Locating Mesiobuccal Canal 2 of the Maxillary 1st Permanent Molar*

Specimen	With Ease (less than 1 min)	With minimal difficulty (less than 3 mins.)	With Difficulty (more than 3 mins.)	Was not able to locate
A	2 (5.0%)	2 (5.0%)	8 (20.0%)	28 (70.0%)
B	27 (67.5%)	9 (22.5%)	4 (10.0%)	0
C	36 (90.0%)	4 (10.0%)	0	0

Table 2. shows that the majority of the respondents were not able to locate the MB2 (70%) specimen A, both the respondents who were able to find the MB2 with minimal difficulty and with ease (5%).

Table 2. shows that the majority of the respondents were not able to locate the MB2 (70%) specimen A, both the respondents who were able to find the MB2 with minimal difficulty and with ease (5%). While specimen B, the majority of the respondents were able to locate the MB2 with ease (67.5%). Lastly, in specimen C, the majority of the respondents were to locate the MB2 with ease (90%).

Table 3. *Difference between the Ease Locating the Mesiobuccal Canal 2 of the Maxillary 1st Permanent Molar using Disclosing Solution and Ophthalmic Dye.*

Specimen	Tabular Value	Degree of Freedom	Computed Value	Decision	Interpretation
A vs B	2.02	39	-11.38	H_0 Rejected H_A Accepted	Significant Difference
A vs C	2.02	39	-17.13	H_0 Rejected H_A Accepted	Significant Difference
B vs C	2.02	39	-3.34	H_0 Rejected H_A Accepted	Significant Difference

Paired T-test

Table 3. shows the comparison of the ease of locating the Mesiobuccal canal 2 of the Maxillary 1st Permanent Molar. At 0.05 level of significance, there were significant differences noted in the comparison of the evaluations. The results suggest that the ease of locating the Mesiobuccal canal 2 of the Maxillary 1st Permanent Molar was found to be easiest using ophthalmic dye solution.

Table 4. A*ssociation of the Demographic Characteristics with Level of Difficulty when Locating Mesiobuccal Canal 2 of the Maxillary First Permanent Molar (Specimen A)*

Specimen	n	With Ease (< 1 min) (n=2)	With minimal Difficulty (< 3 mins.) (n=2)	With Difficulty (> 3 mins.) (n=8)	Was not able to locate (n=28)	*p-value
Age (in years)						
21 – 23	18	1 (50.0%)	1 (50.0%)	5 (62.5%) 0	11 (39.3%)	
24 – 26	9	1 (50.0%)	0	3 (37.5%)	8 (28.6%)	
27 – 30	5	0	0	0	2 (7.1%)	0.29 (NS)
31 – 35	5	0	1 (50.0%) 0	0	4 (14.3%)	†
36 – 38	3	0			3 (10.7%)	
Clinic Level						
2	10	0	0	4 (50.0%)	6 (21.4%)	
3	14	1 (50.0%)	1 (50.0%)	2 (25.0%)	10 (35.7%)	0.64 (NS)
4	16	1 (50.0%)	1 (50.0%)	2 (25.0%)	12 (42.9%)	†
Sex						
Male	14	1 (50.0%)	1 (50.0%)	1 (12.5%)	11 (39.3%)	0.49 (NS)
Female	26	1 (50.0%)	1 (50.0%)	7 (87.5%)	17 (60.7%)	†

** p>0.05- Not significant; p ≤0.05 -Significant*

† Chi-square test

Table 4 shows that age, clinic level, and sex were not significantly associated with the level of difficulty as proven by all p values >0.05.

Table 5. *Association of the Demographic Characteristics with Level of Difficulty when Locating Mesiobuccal Canal 2 of the Maxillary First Permanent Molar (Specimen B)*

Specimen	n	With Ease (< 1 min) (n=27)	With minimal Difficulty (< 3 mins.) (n=9)	With Difficulty (> 3 mins.) (n=4)	*p-value
Age (in years)					
21 – 23	18	12 (44.4%)	4 (44.4%)	2 (50.0%)	
24 – 26	9	6 (22.2%)	2 (22.2%)	1 (25.0%) 0	
27 – 30	5	5 (18.5%)	0	1 (25.0%) 0	0.80 (NS)†
31 – 35	5	2 (7.4%)	2 (22.2%)		
36 – 38	3	2 (7.4%)	1 (11.1%)		
Clinic Level					
2	10	8 (29.6%)	1 (11.1%)	1 (25.0%)	
3	14	7 (25.9%)	6 (66.7%)	1 (25.0%)	0.27 (NS)†
4	16	12 (44.4%)	2 (22.2%)	2 (50.05)	
Sex					
Male	14	11 (40.7%)	1 (11.1%)	2 (50.0%)	0.22 (NS)†
Female	26	16 (59.3%)	8 (88.9%)	2 (50.0%)	

** p>0.05- Not significant; p ≤0.05-Significant*
† Chi-square test

Table 5 shows age, clinic level, and sex were not significantly associated with the level of difficulty as proven by all p values >0.05.

Table 6. *Association of the Demographic Characteristics with Level of Difficulty when Locating Mesiobuccal Canal 2 of the Maxillary First Permanent Molar (Specimen C)*

Specimen	n	With Ease (< 1 min) (n=36)	With minimal Difficulty (< 3 mins.) (n=4)	*p-value
Age (in years)				
21 – 23	18	17 (47.2%)	1 (25.0%)	
24 – 26	9	8 (22.2%)	1 (25.0%)	
27 – 30	5	5 (13.9%)	0	0.50 (NS)†
31 – 35	5	4 (11.1%)	1 (25.0%)	
36 – 38	3	2 (5.6%)	1 (25.0%)	
Clinic Level				
2	10	9 (25.0%)	1 (25.0%)	
3	14	13 (36.1%)	1 (25.0%)	0.89 (NS)†
4	16	14 (38.9%)	2 (50.0%)	
Sex				
Male	14	12 (33.3%)	2 (50.0%)	0.60 (NS)‡
Female	26	24 (66.7%)	2 (50.0%)	

** p>0.05- Not significant; p ≤0.05-Significant*
† Chi-square test; ‡ Fisher Exact test

Table 6 presents that age, clinic level, and sex were not significantly associated with the level of difficulty as proven by all p values >0.05.

DISCUSSION

The study utilized a quantitative, quasi-experimental method of research including 40 participants from 6th-year clinicians enrolled in S.Y. 2019-2020 in a private institution in Caloocan City, who completed their pre-clinical subject requirements in Endodontics. The participants located the mesiobuccal canal 2 of the maxillary 1st permanent molar of the three prepared specimens marked as A: without any staining solution; B: with disclosing solution and C: with Ophthalmic dye.

According to the age of the respondents, the majority of them were in the range of 21-23 years of age (45%). This signifies that the majority of the respondents started their school of dental medicine within the year 2012 to 2014. Since the course of dentistry is a six-year course, the study indicated that most of the respondents can be considered regular students in dentistry. While the remaining percentage is above the age level of the majority because the respondents were either retained or second-course takers.

As to the gender of the participants, the results were shown in Table 1.2 state that the majority of the respondents were female (65%). Results showed that there is no significant relationship between the respondent's gender and ease of locating the Mesiobuccal Canal 2 of the Maxillary 1st Permanent Molar as proven by all p values >0.05. According to Ali, Subhi, Ringsted, and Konge (2015), gender differences do not affect clinical skills.

With regards to the clinic level, there are 25% respondents on the clinic level 2 while 35% were in the clinic level 3. Though the percentages of clinic level 2 and 3 were lower than the percentage of clinic level 4, the result of the study indicated that not all the respondents reached the standard clinic level on the 6th year due to delays in clinical requirements.

With the Level of Difficulty when locating Mesiobuccal Canal 2 of the Maxillary 1st Permanent Molar, the majority of the respondents (70%) found it very difficult to locate the canal on specimen A; without staining solutions. However, the respondents found both the Disclosing Solution (67.5%) and Ophthalmic Dye (90%) with ease in locating the canal as results shown in Table 2.

According to the study of R. Signori (2019), in endodontics, the maxillary first permanent molars face a constant challenge, given the difficulty of finding and treating the fourth canal, called the mesiobuccal canal 2 or MB2, which begins at a steep mesial slope from the pulp chamber and then bends back distally, making it hard to locate and treat.

With the differences between the ease of locating the Mesiobuccal Canal 2 of the Maxillary 1st Permanent Molar using Disclosing Solution and Ophthalmic Dye, there is a significant difference between specimen A; with no staining solution and B; with Disclosing Solution, specimen A; with no staining solution and C; with Ophthalmic Dye and specimen B; with Disclosing Solution and C; with Ophthalmic Dye. Both dyes worked and can be used as a staining solution to locate the MB2. However, at 0.05 level of significance, there were significant differences and the results suggest that the ease of locating the Mesiobuccal canal 2 of the Maxillary 1st Permanent Molar was found to be easiest using Ophthalmic Dye solution as results shown in Table 3.

Cited from the article of Ghandi et. al., (2019), 62% of the times that the operators were at ease in locating the canal of vital primary molars and they are satisfied in using the ophthalmic dyes (fluorescein sodium) in locating the canal orifices as compared with the unaided eye and use of magnifying loupes.

Based on the results shown in Table 4 in Association of the Demographic Characteristics with Level of Difficulty when Locating Mesiobuccal Canal 2 of the Maxillary First Permanent Molar for (Specimen A), Table 5 for (Specimen B) and Table 6 for (Specimen C), the age, clinic level, and sex were not significantly associated with the level of difficulty as proven by all p values >0.05. Because each of the respondents already finished their pre-clinical subject in Endodontics, in which they have already acquired the cognitive skills in locating the mesiobuccal canal 2 of the maxillary first permanent molar.

Academic status and pre-clinical training were both very essential in the development of clinical proficiency. In this stage, the development of manual dexterity and comprehension of procedures are simulated procedures on dental procedures. Improvement of performance is the result of the repetition of the procedure (Velayo, Starcj, Eisen & Kugel, 2014).

Limitations of the Study

This research was conducted to assess the effectiveness and comparison of these dyes in locating the MB2 canal. The limitation of the study was only on the three (3) specimens of Maxillary 1st Molars either extracted or bought from the dental store and was prepared by the researchers. The specimens were already prepared before the participation of the respondents. Each specimen was embedded on the ivory wax.

The three (3) prepared specimens were placed on the table marked as A, control specimen; B, a specimen with disclosing solution; and C, a specimen with ophthalmic dye. Given that the specimens were not fractured tooth, a tooth with restorations, incomplete root formation, and history of previous root canal treatment.

Conclusion

Proper location and treatment of the canals are essential for the success of root canal treatment. It can be concluded that the disclosing solution and ophthalmic dye are effective to aid in locating the mesiobuccal canal 2 of the maxillary 1st permanent molar in the absence of endodontic loupes and endodontic microscope.

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Optimum Washing Soda Ratio And Developing Time Of Caffenol For Dental Radiography

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ABSTRACT

Introduction: Instant coffee contains phenols to potentially develop radiographic film and washing soda accelerates this process. This study aims to determine the optimum washing soda ratio and developing time that will produce an image quality relative to the commercially available developing solution. ***Methodology:*** In the first phase, ratio-time combinations that passed the criteria were chosen. Four ratios and three developing times were visually scrutinized based on the distinguishability of dental tissues, surrounding structures, and anatomical landmarks. In phase two, different ratio-time combinations were used to determine the optimum. The films were digitized and RGB values were measured from four selected areas within and around tooth 11. One sample t-tests were performed to compare the caffenol to the conventional group. ***Results:*** Films developed in ratio 1:2, 1:3 (caffenol-washing soda) at 150, 180, and 210 seconds contained distinguishable radiographic landmarks. Phase 2 obtained no optimal ratio-time combination for enamel and PDL Space. For root dentin, B180 had a p-value of 0.157 while for the alveolar bone, B180 and C210 both had p values (0.163 and 0.394 respectively). At these ratio-time combinations, the color densities of the caffenol group did not significantly differ from those of the conventional group. ***Conclusion:*** There was no uniform optimum combination of ratio and time that was obtained. Immersion of radiographic films in a caffenol-washing soda ratio of 1:3 for 180 seconds produced color densities closest to those of conventionally developed films in the areas of root dentin and alveolar bone.

Keywords: Caffenol, RGB values

INTRODUCTION

Background

Roentgenology is a branch of medical science that uses radioactive substances and radiant energy to help in diagnosis and treatment of diseases. [1] It covers several types of studies that visualize the internal parts of the body through x-rays, discusses the components of a developing and fixing solution, and evaluates a good quality radiograph.

In dentistry, dental radiographs are used to evaluate the teeth and their surrounding structures, including prostheses and dental restorations. This can help dentists identify problems, like caries, lesions, and impacted teeth, do proper case selection, treatment, and prognosis. Only low levels of radiation are used for dental purposes, where bitewing radiographs is only 0.005mSv every 4 exposures. [2]

In processing conventional radiographs, there are two methods: automatic processing and manual processing. Both entail the use of a developer and fixer to affix the image on the film. From the two, manual processing is usually used in dental clinics since a machine is not needed to do the process although the processing must be performed properly in order to obtain clinically acceptable radiographs.

The conventional developing agent has an active ingredient, phenol in the form of hydroquinone. Hydroquinone is a type of phenol derivative widely used as a skin bleaching agent, to lighten darker areas where there are freckles, melasma, age spots, and acne scars. Only small concentrations of this compound should be used in creams— 2% [3] and 4% is only available in prescription. [4] Aside from its bleaching properties, it is also used as a reducing agent in conventional developing solutions for radiography and photography comprising at least 5-10% by weight of the whole developing solution. [5] It is responsible for giving out electrons that are used to convert silver halide grains into metallic silver to form a visible image. The key characteristic of a developer is it acts rapidly on the parts of the film exposed to light. The more the film is submerged into the developer, the darker the image formed but this may also affect the areas where no light has penetrated.

There have been some studies exploring the possible carcinogenic effect of hydroquinone in the long term because medium-term effects like white patches on the skin and subcutaneous dark pigment have been seen in patients. This compound can cause permanent damage to the DNA cells and inhibit the apoptosis of mutated cells. [6] Further research on this possibility is implored. When in a developing solution, it is advised that hydroquinone be kept away from eyes, skin due to possible irritation, not be ingested or inhaled because it might cause lung edema and lung damage from the phenol which is absorbed rapidly in the lungs. [5]

A safe and economically sound alternative to hydroquinone as a developing agent would be coffee or more specifically instant coffee. Instant coffee is rich in phenolic acids which have the potential to be an excellent reducing agent used in developing solutions.

It contains many kinds of phenolic acids and derivatives, most abundantly chlorogenic acid (CGA) which is a mix of caffeic acid and quinic acid. her compounds such as tannins, lignans, and anthocyanins are also present but in smaller amounts. These compounds have exhibited significant antioxidant, hypoglycemic, antiviral, hepatoprotective, and antispasmodic activities. [7]

Caffenol has been primarily used as a photographic film developer since its discovery by the Rochester Institute of Technology in 1995 in its pursuit to identify nontraditional developers. From the research, it was concluded that tea and coffee could develop photographic films because they are rich in phenolic acids called tannins. Though images were evident in both solutions, coffee had better development.

Caffenol has four components: washing soda (WS), coffee crystals (C), vitamin C (Vc) powder, and distilled water (W) which can produce a low-contrast and soft negative image. Each of these components acts as an alternative to the components present in the conventional developing solution. It is considered safer for both the environment and the operator who is handling the solution since it does not deal with potentially hazardous chemicals like hydroquinone, it is also inexpensive and much more accessible because all the ingredients are readily available. Though if not properly prepared and processed, caffenol could develop the film in a sepia tone across all the negatives because of its natural brown color that will potentially stain the film.

There has been little to no scientific research about the optimum ratio of caffenol components as well as the optimum time required for processing. This is what this research aims to study and explore through its experimental nature. Through the process of elimination during pilot test runs, from four different ratios and three different times, it was narrowed down to three ratios and two times.

METHODOLOGY

Phase 1

Twelve films were processed in the first phase of this study. The following ratios were used: 1:1, 1:2, 1:3, 1:4 (1 being all other components of caffenol - instant coffee, vitamin C, and water and the washing soda is increased in each ratio) and each ratio having three different developing times: 150s, 180s, 210s.

The components of caffenol were mixed in smaller amounts but with the same concentration. There were 4 beakers with 100mL of water and instant coffee amounting to 4 grams, vitamin c 1.6 grams, and washing soda 5.4 grams, 10.8 grams, 16.2 grams, and 21.6 grams respectively.
After the film processing and drying, the film was placed on the negatoscope and the elimination process was done.

The criteria for elimination were: distinguishability of dental tissues (DT), surrounding structures (SS) only on and around tooth 11 since it is the area of interest for phase 2, and maxillary radiographic landmarks (MRL). All these will be taken into account when scrutinizing the films.

Phase 2

In the second phase, a total of 121 sample films were used, 1 film was developed using the conventional developing solution, and for the rest, there were 20 films used per set-up for different ratios and time combinations. A modified paralleling device with a bite registration and a border for the film made out of self-cured resin was placed onto a random skull for constant bite positioning and uniform placement of films.

After all, radiographs have been processed, they were digitized using a DSLR camera and were analyzed through Adobe Photoshop. The RGB values of the selected four areas were measured individually and were tabulated per group to compare the RGB values.

Preparation of the Caffenol

Three different beakers labeled A (1:2), B (1:3), and C (1:4) were used with 54, 81, and 108 grams of washing soda respectively and a fixed amount of the following: 8 grams of vitamin C, 20 grams of coffee, and 500mL of water each beaker. The contents were stirred with a stirring rod for 5 minutes. A thermometer was used to determine the initial temperature of the solution and a cold, water bath was used to bring it down to 20 degrees Celsius. Also, a pH paper was used to check the pH of the solution, making sure that it is between the range of 10-11. Each setup was divided into 20 containers containing 25 mL of the solution.

Preparation of film placement for x-ray procedure

A random skull was used as a model for this experiment. The paralleling device used was modified using self-cured resin to create definite borders for proper bite registration and exact film placement during numerous film exposures.

Developing the films

From the total amount of 120 experimental films, 20 films were used per setup. Developing times are as follows: 180 seconds and 210 seconds. Post-developer processing times were 60 seconds in running water, submerged in the fixer for twice the developing time, and lastly, washed in running water for 60 seconds. Films were left in film hangers to air dry.

Digitization of the Films

Films were digitized per group using a Canon DSLR to capture the film and will be run through Adobe Photoshop.

The DSLR camera setting will be set as follows: ISO 800, 1/125th seconds of shutter speed, aperture of f10, black and white, and flash of the camera was turned off. The position of the DSLR camera was stabilized by a tripod. A thin rigid plastic (4x4in.) was cut at the center to create a space that would exactly fit the size of the film, this plastic was placed in the negatoscope to maintain the position of the film. The negatoscope was placed against a wall on top of a stool and there was a 12 cm distance from the film to the camera lens. Also, the parts of the negatoscope not being used were covered with black cartolina to prevent light leakage that will alter the digitization process.

RESULTS AND ANALYSIS

Phase 1

In phase one of this study, visual scrutiny was used to eliminate those films which did not present acceptable visualization of dental tissues and anatomical landmarks. Criteria evaluated were distinguishability of dental tissues (DT), surrounding structures (SS), and maxillary radiographic landmarks (MRL) like the incisive canal, intermaxillary suture.

Table 1. *Summary of Visibility of Dental Tissues (DT), Surrounding Structures (SS) and Maxillary Radiographic Landmarks (MRL) for 4 ratios in 150, 180, and 210 seconds*

RATIO	TIME (SECONDS)	DENTAL TISSUES (DT), SURROUNDING STRUCTURES (SS), AND MAXILLARY RADIOGRAPHIC LANDMARKS (MRL)	
		IS DISTINGUISHABLE	IS NOT DISTINGUISHABLE
1:1	150		✔
	180		✔
	210		✔
1:2	150		✔
	180	✔	
	210	✔	
1:3	150		✔
	180	✔	
	210	✔	
1:4	150		✔
	180	✔	
	210	✔	

The table above shows that films developed for in ratio 1:1 (CVcW:WS) was the one that did not meet the criteria for evaluation because they did not contain any distinguishable DT, SS and MRL also, all films developed for 150 seconds in any of the four ratios did not possess any image that can be visualized. All films from this time (segment) were of low density. Therefore, there was no distinguishable DT, SS, and MRL. Most of the images on the film appeared white. Films from these 2 variables, 1:1 ratio, and 150 seconds were excluded from the phase 2 experiment.

Phase 2

In phase two, quantitative data was gathered through Adobe Photoshop to obtain grayscale values in the form of RGB values as read by the software. The mean and standard deviation of the RGB values of the conventional will be seen in Table 2. Areas of interest, specifically in enamel, root dentin, periodontal ligament, and alveolar bone on and around tooth 11 were measured and averaged. Averages of each area per ratio and time are illustrated in table 2.1, 2.2, 2.3, and 2.4.

Table 2. *Mean and Standard Deviation for Conventional*

Conventional	Mean	Standard Deviation	Median	Pixels
Area 1	174.91	9.97	177	3,364
Area 2	188.67	5.49	189	3,364
Area 3	157.85	6.57	158	14,200
Area 4	178.5	6.82	179	56,169

Table 2.1. *Mean and Standard Deviation Per Ratio and Time for Area 1 (Enamel)*

	N	Mean	Std. deviation
A180_A1	20	191.02	7.35
A210_A1	20	198.04	4.78
B180_A1	20	178.98	8.62
B210_A1	20	194.75	8.91
C180_A1	20	191.56	9.71
C210_A1	20	172.50	4.65
B210_A1	20	191.02	7.34

Table 2.2. *Mean and Standard Deviation Per Ratio and Time for Area 2 (Root Dentin)*

	N	Mean	Std. deviation
A180_A2	20	199.21	5.66
A210_A2	20	206.73	3.92
B180_A2	20	191.18	7.60
B210_A2	20	204.32	6.85
C180_A2	20	199.19	6.10
C210_A2	20	185.62	3.97

Table 2.4. *Mean and Standard Deviation Per Ratio and Time for Area 4 (Alveolar Bone)*

	N	Mean	Std. deviation
A180_A4	20	192.31	5.34
A210_A4	20	199.52	5.50
B180_A4	20	181.05	7.85
B210_A4	20	196.89	7.62
C180_A4	20	191.80	7.43
C210_A4	20	177.59	4.67

For area 1 (enamel), the ratio and time of A210 gave the highest mean and lowest standard deviation (M=198.04, SD=4.78) and the ratio and time combination of B180 gave the closest mean to that of the conventional RGB values.

For area 2 (root dentin), the ratio and time of A210 gave the highest mean and lowest standard deviation (M= 206.73, SD= 3.92) and the ratio and time of B180 gave the closest mean to that of the conventional RGB values.

For area 3 (PDL space), the ratio and time of A210 gave the highest mean and lowest standard deviation (M=192.49, SD= 11.87) and the ratio and time of C210 gave the closest mean to that of the conventional RGB values.

For area 4 (alveolar bone), the ratio and time of A210 gave the highest mean (M= 199.52, SD= 5.50) and the ratio and time C210 gave the lowest mean (M=177.59, SD=4.67) and the lowest standard deviation (4.67). However, the ratio and time combination of B180 gave the closest mean to that of the conventional RGB values.

Table 3.1. *Comparison of Caffenol group to the Conventional group for Area 1 (Enamel) using the one sample t-test*

Area 1	P value
A180_A1	<.001
A210_A1	<.001
B180_A1	.048
B210_A1	<.001
C180_A1	<.001
C210_A1	.032

Table 3.2. *Comparison of Caffenol group to the Conventional group for Area 2 (Root Dentin) using the one sample t-test*

Area 2	P value
A180_A2	<.001
A210_A2	<.001
B180_A2	.156
B210_A2	<.001
C180_A2	<.001
C210_A2	.003

Table 3.3. *Comparison of Caffenol group to the Conventional group for Area 3 (PDL Space) using the one sample t-test*

Area 3	P value
A180_A3	<.001
A210_A3	<.001
B180_A3	<.001
B210_A3	<.001
C180_A3	<.001
C210_A3	<.001

Table 3.4. *Comparison of Caffenol group to the Conventional group for Area 4 (Alveolar Bone) using the one sample t-test*

Area 4	P value
A180_A4	<.001
A210_A4	<.001
B180_A4	.163
B210_A4	<.001
C180_A4	<.001
C210_A4	.394

For area 1 (enamel), there is a significant difference, which means that there is no optimal ratio and time combination obtained from the said area relative to the RGB values of the conventional.

For area 2 (root dentin), the ratio and time of B180 have the highest p-value of 0.157 > p-value which means that there is no significant difference between the values of B180 and the conventional for this area.

For area 3 (PDL space), there is also no optimal ratio and time combination obtained from the said area relative to the RGB values of the conventional.

For area 4 (alveolar bone), the ratio and time of B180 and C210 both have p values (0.163 and 0.394 respectively) > than p-value, meaning these two ratio and time combinations are the closest to the conventional reading for this area.

The following are ratios of the areas that gave the closest mean value relative to the conventional: for area 1=B180, area 2 = B180, area 3= C210, and area 4= B180. However, there was no optimal ratio and time combination obtained for areas 1 and 3.

DISCUSSION

In a study conducted whose purpose was to know the efficacy of caffenol in developing radiographs, as well as lowering the health risks of the dentist or student when processing images, it was concluded that caffenol caPDL be comparable to the conventional if developed in 5 minutes, washed for 1 minute and fixed for 5 minutes. [8] On the other hand, adding washing soda with instant coffee makes the solution alkaline to make a suitable level for the developing agents to operate as well as to accelerate the process of developing. [9]

Instant coffee contains phenols which can be the reducing agent in developing films, and it also contains the substance called chlorogenic acid (CGA) which is a mixture of caffeic acid and quinic acid. The abundance of chlorogenic acid in different pre-ground coffee and instant coffee were analyzed using HPLC (high-performance liquid chromatography) and results were that pre-ground coffee CGA contents ranged from 27.33- 94.47 mg/200mL while instant coffee ranged from 37.04-101.25mg/200mL with the coffee green variant having the highest CGA content. [10]

The two of the main reasons instant coffee was chosen over ground coffee beans are the consistency of the CGA content with only one outlier, the green variant, and the high low, and maximum values the research stated.

Phenols reacting with the exposed silver halide crystals in the film is the primary reaction of the developing process. Washing soda acts as the accelerator for the process, softening the emulsion layer of the film allowing the phenol to more effectively penetrate the sensitive sites of the silver halide. Without an accelerator, the process will still happen but at a much slower rate. In theory, the more accelerator, the softer the emulsion the more rapid and more efficient the reaction. This is the determining factor of the process. Though when caustic alkalis like sodium hydroxide and potassium hydroxide are used, the developers produce films with high contrast these swell the gelatin excessively causing the silver halide grains to clump together producing larger grains creating a non-fine grained image. Also, an excess in the alkali component will result in eventual chemical fogging because it will attack even the unexposed crystals creating a low contrast image. Chemical fogging also results if the solution is immersed in the solution for more than the allowed time for developing as well as the lack of action of the restrainer. Though there is a natural restrainer that is made from the halides released when the phenols react with the silver from the silver halide, sometimes it is still not enough which is why conventional developers have an added restrainer because the action of the hydroquinone is too potent as well as they use caustic alkali. The absence of this said restrainer in the caffenol created lower levels of contrast in the various areas studied. In addition, the inorganic component of these hard tissues which consists of biological apatite, Ca10(PO4)6(OH)2, the enamel has more inorganic content approximately 90% prismatic crystals than dentin and bone which consist of approximately 70%, and cementum with 45% [11] Due to this reason the area 2 (root dentin) and 4 (alveolar bone) which has the closest biological apatite resulted in acquiring the optimum ratio and time combination of caffenol as relevant to that of the conventional developing solution. Area 1 (enamel) and 3 (PDL space) showed a significant difference with the conventional radiographic image due to the distinct difference to its biological apatite, and because of the caffenol's shortcoming by its lack of restrainer.

The result that was obtained through the t-test was that the RGB values of the landmarks (enamel, dentin, PDL space, and alveolar bone) produced different ratio and time combinations that were close to the RGB values acquired from the conventional developing solution. The higher the RGB values, the whiter the image produced while the lower these values are, the darker image was produced.

Researchers utilized size 2 D- speed periapical films. Only the maxillary incisor area of one skull was exposed all throughout and a paralleling device was used. Oral pathologies and other radiolucent areas found were not included in this study and were focused on maxillary anatomical landmarks and dental tissues. The default x-ray machine settings – 60 kvp, 7 mA for 0.160 seconds, and beam angulation were kept constant. For the caffenol, only the amount of washing soda varied in each ratio, all other components remained the same.

A DSLR (digital single-lens reflex) camera was used to digitize the film a few hours after they have been completely air-dried. Uniform settings of the camera (800 ISO and 1/125 shutter speed) were also achieved.

The shelf life of both caffenol and film as well as the long-term effect of the caffenol on the film were excluded in this study. Due to time constraints, only three ratios versus two different developing times were tested.

Conclusion

There was no uniform optimum combination of ratio and time that was obtained in this experiment. Immersion of radiographic films in a caffenol-washing soda ratio of 1:3 for 180 seconds produced color densities closest to those of conventionally developed films in the areas of root dentin and alveolar bone.

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The Anti-inflammatory Activity of Sabila (Aloe vera) Gel on White Mice (Mus musculus) Albumin-induced Paw Edema

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ABSTRACT

Introduction: Aloe vera is widely used in skincare, cosmetics, and as nutraceuticals. The research process includes collection and preparation of Aloe vera plant, formulation of gel, preparation of animal groups, weighing, and measuring. ***Methodology***: The area injected was disinfected with ethyl alcohol before acute inflammation, then it was induced by injecting 0.20 ml of 1% albumin at the sub-plantar surface of the left hind paw of white mice to produce egg albumin-induced inflammation. The experiment process includes topical application of gel treatments and monitoring of edema. The descriptive data include mean and standard deviation on the paw thickness and percent inhibition of mice paw edema after the treatment. Different treatments to various groups such as positive control (commercial ointment), experimental group (aloe vera ointment), and negative control (petroleum jelly) were applied. ***Results and Conclusion***: The most reduced paw size was present in the positive control while the least reduced was in the negative control group. It showed a significant difference in the paw size in various time intervals from the induction of inflammation to the treatment over a 6-hour monitoring period. Each treatment used over a 6-hour period showed a significant difference within the treatment used. The positive control is the most effective followed by the experimental group but not of the negative control. The study showed a significant difference in the paw size in the various time intervals from the induction of inflammation to the treatment over a 6 hour monitoring period, f (62.642), p (0.000) < 0.05. The effect size is large (0.952) which means that the effect is due to the treatment used. There is also a significant interaction between the group and time of monitoring on the paw size, f (2.578), p (0.013) < 0.05. The effect size is large (0.449) which means that the effect is due to the treatment used.

INTRODUCTION

Inflammation is sometimes associated with something bad but it is not always the case, it is actually part of the body's healing process. Inflammation can occur in various parts of the body and it could be due mainly to two reasons. First is the way toward warding off remote bodies like infections and microbes, which assault our safe framework by synthetic compounds and white platelets and is a characteristic reaction and genuinely necessary. Second is an immune system sickness, which causes a provocative reaction not

withstanding when there are no outside bodies to fend off. Studies have shown that Aloe Vera contains 75 potentially active constituents: vitamins, enzymes, minerals, sugars, lignin, saponins, salicylic acids, and amino acids. Vitamins: It contains vitamins A (beta-carotene), C, and E, which are antioxidants. It also contains vitamin B12, folic acid, and choline. A novel anti-inflammatory compound called C-glucosyl chromone was isolated from gel extracts of fresh Sabila (Aloe Vera) and it significantly reduced acute inflammation in rats (carrageenan-induced paw edema), but not in chronic inflammation. It has also been reported that the aloe sterol includes campesterol, β-sitosterol, lupeol, and cholesterol which are anti-inflammatory in nature, helps in reducing inflammation pain, and act as a natural analgesic. Other aspirin-like compounds are also present in Aloe Vera are responsible for anti-inflammatory and antimicrobial properties. It is assumed that Aloe Vera could help in treating periodontitis or oral inflammation in particular or the inflammatory reaction in the surrounding tissues of the tooth, thus, the focus of this study.

METHODOLOGY

The study involved 3 treatments: Treatment A (Positive Control: Commercial Anti-inflammatory Gel), Treatment B (Negative Control: Petroleum Jelly), and Treatment C (Experimental treatment: Sabila gel).

The materials utilized in this study were the following: Approximately 5 kilograms of Sabila Plant, Blender for cutting the Sabila into pieces, 2 liters of solvent (ethanol), rotary evaporator, petroleum jelly for the formulation of the gel, 1ml syringe for inducing albumin, cotton swabs for ointment application, Vernier caliper for measuring the paw size and ethyl alcohol for disinfecting the part of mice to be injected.

Preliminary Activities

Materials needed were collected and prepared in advance before the start of the experiment.

Collection and Preparation of Sabila (Aloe vera) Plant

The Sabila plant was air-dried for 2 days. About 3 kilograms of blended Sabila has been blended and soaked in 2 liters of ethanol solvent for 48 hours. After soaking, it was filtered to separate the plant residues and the filtrate. The filtrate was then rotary evaporated for 3 days until extract will be obtained. The extract was used in the formulation of the gel.

Formulation of Gel

Twenty grams of petroleum jelly was weighed and combined with 20 ml of Sabila extract with vigorous stirring and application of medium heat through a water bath. Tween-80 was added also to the mixture, this chemical will allow all parts of the extract and petroleum jelly to be mixed thoroughly. The prepared gel was transferred to a clean jar and stored at room temperature.

Preparation of Animal Groups

A total of twenty-seven (27) male white mice ranging from 17-39 grams were procured by the researchers from the animal breeder from Delgado St., Iloilo City. The mice were kept in a wood cage, fed with commercially available standard grower feeds and water, and maintained under standard laboratory conditions. The mice were acclimatized for 1 week prior to the experiment proper.

The mice were handled in accordance with the protocol approved by the IACUC (Institutional Animal Care and Use Committee) and Department of Agriculture Administrative Order Number 40. The mice used were divided into 3 groups each and of the same sex (All males). This was done in 3 trials with 3 replicates each. The people who handled the animal during the experiment were licensed and had undergone training in animal handling.

Weighing and Measuring

Prior to the experiment proper, the mice paw initial weight was measured. The paw thickness in control/treatment was measured using a Vernier caliper.

Experiment Proper

Egg Albumin-Induced Inflammation

The area injected was disinfected with ethyl alcohol before acute inflammation and it was induced by injecting 0.20ml of 1% albumin at the sub-plantar surface of the left hind paw of white mice. After the usage of albumin, it was then kept and stored in a refrigerator. Two hours after the administration or application of the treatment, the mice were given access to food and water.

Topical Application of Gel Treatments

All treatments were applied topically 30 minutes after the induction of inflammation and measurement of paw size induced edema. One group received petroleum jelly (placebo), one group received the commercial anti-inflammatory gel and the last group received the Sabila gel. All treatments were applied in 3 groups of triplicates at 1mg per square centimeter of the inflamed area of the skin.

Monitoring of Edema

Edema was assessed every 1-hour interval. The thickness of the left hind paw was repeatedly measured using a Vernier caliper. The average foot swelling was compared to the negative control, positive control, and experimental.

Data Collection Procedure

The data collected was based on paw thickness with the formula:

a. Paw thickness. The increase in paw thickness in control/treatment was calculated using the formula. Increase in paw thickness Control or Treatment group= Pt-Po, Where Pt= paw thickness at time t, Po=initial paw thickness.

b. Percent Inhibition. The percent inhibition (anti-inflammatory activity) of edema was determined using the formula at the end of the 7-hour period. Percent inhibition is where A minus B is divided by A times 100. Where A is edema thickness of the negative control and B is edema of the experimental or positive control groups.

Waste Disposal

The mercy killing procedure was done after the experiment proper. Proper disposal of waste was using leak-proof plastic bag then final disposal was destroyed in the hazardous animal treatment plant.

Data Analysis

The data collected was subjected to both descriptive and inferential analysis.

Descriptive Data Analysis

It includes the mean and standard deviation on the paw thickness and percent inhibition of mice paw edema after the treatment. These were further plotted in a line graph for paw thickness while bar graph for percent inhibition.

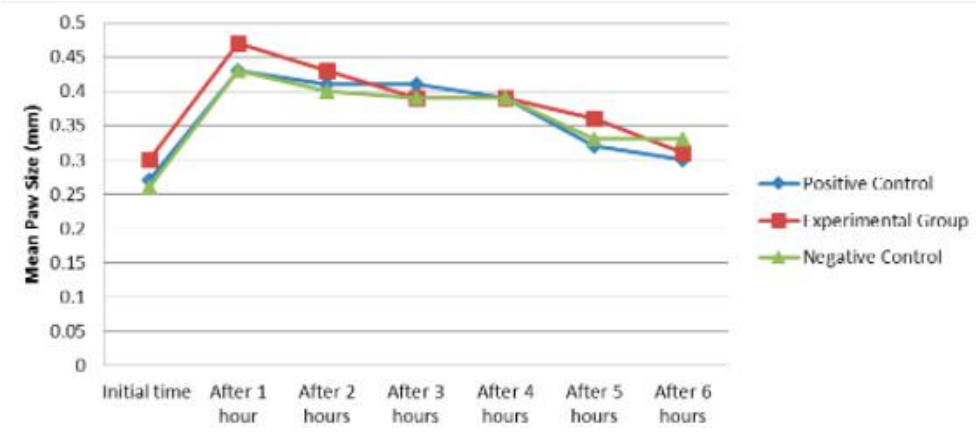
Inferential Data Analysis

Repeated measures of ANOVA (rANOVA) were used to assess any significant differences in the paw thickness and percent inhibition of mice paw edema over the 7-hour period of treatment. The inferential results were evaluated at a 0.05 level of significance.

RESULTS AND ANALYSIS

Figure 2 presents the graphical presentation of the mean paw over a 6-hours monitoring period on the Anti-Inflammatory effects of Aloe Vera Ointment Leaf Extract on Albumin-Induced Paw Edema in White Mice. It can be seen that the commercial ointment (Positive Control) showed a significant difference in the paw size followed by the Aloe Vera ointment (Experimental Control) and the Petroleum Jelly (Negative Control) in the various time intervals from the induction of inflammation to the treatment over a 6-hour monitoring period.

Figure 2. *Mean paw over a 6-hours monitoring period on the anti-inflammatory effects of Aloe Vera Ointment Leaf Extract on Albumin-Induced Paw Edema in White Mice*



In the initial time, the paw size diameter in each group is less similar in size. After 1-hour induction of inflammation, the paw size diameter increased which was considered to be inflamed. These were then treated with different treatments to various groups as positive control (commercial ointment), experimental group (Aloe Vera ointment), and negative control (petroleum jelly). After an hour until the 6th hour, all paw sizes in the three groups subsided. The most reduced paw size was present in the positive control while the least reduced one was in the negative control group.

Table 1. Multivariate Tests on the Anti-Inflammatory effects of Aloe Vera Ointment Leaf Extract on Albumin-Induced Paw Edema in White Mice

Effect		Value	f	Hypothesis df	Error df	Sig.	Partial Eta Squared
Time	Wilks' Lambda	.048	62.641	6.000	19.000	.000	.952
Time * Group	Wilks' Lambda	.304	2.578	12.000	38.000	.013	.449

Note: *P <0.05 is significant. Effect Size: 0.01 = small; 0.06= medium; 0.14= large

Data in Table 1 shows the multivariate tests on the anti-inflammatory effects of Aloe Vera ointment leaf extract on albumin-induced paw edema in white mice. It shows a significant difference in the paw size in the various time intervals from the induction of inflammation to the treatment over a 6 hour monitoring period, f (62.642), p (0.000) < 0.05. The effect size is large (0.952) which means that the effect is due to the treatment used. There is also a significant interaction between the group and time of monitoring on the paw size, f (2.578), p (0.013) < 0.05. The effect size is large (0.449) which means that the effect is due to the treatment used.

Table 2. *Tests of Within-Subjects Contrasts on the Anti-Inflammatory effects of Aloe Vera Ointment Leaf Extract on Albumin-Induced Paw Edema in White Mice*

Source	Time	Type III Sum of Squares	df	Mean Square	f	Sig.	Partial Eta Squared
Time	Linear	.017	1	.017	14.527	.001	.377
Time * Group	Linear	.008	2	.004	3.314	.054	.216
Error(Time)	Linear	.028	24	.001			

Note: *P <0.05 is significant. Effect Size: 0.01 = small; 0.06= medium; 0.14= large

Table 2 shows the tests of within-subjects contrasts on the anti-inflammatory effects of Aloe Vera ointment leaf extract on albumin-induced paw edema in white mice. There is a significant difference in the reduction of paw size within the subjects, f (14.527), p (0.001) and interaction of time and group, f (3.314), p (0.054), < 0.05. This means that each treatment used as monitored over a 6-hour period showed a significant difference within the treatment used. Both effect sizes for the time (0.377) and time*group interaction (0.216) were large revealing that the effects are due to the treatments used.

Paw volume was measured before and 4 hours after carrageenan by a mercury plethysmograph and percent increase in paw volume were calculated and used as an index of inflammation. Data are mean ± S.E.M. of six rats per group. * P<0.05 and ** P<0.01 compared to the control group (Hajhashemi et al, 2012).

Table 3. *Tests of Between-Subjects Effects on the anti-inflammatory effects of Aloe Vera Ointment Leaf Extract on Albumin-Induced Paw Edema in White Mice*

Source	Type III Sum of Squares	df	Mean Square	f	Sig.	Partial Eta Squared
Intercept	25.359	1	25.359	19825.410	.000	.999
Group	.010	2	.005	4.052	.030	.252
Error	.031	24	.001			

Note: *P <0.05 is significant. Effect Size: 0.01 = small; 0.06= medium; 0.14= large

Table 3 shows the tests of between-subjects effects on the anti-inflammatory effects of Aloe Vera ointment leaf extract on albumin-induced paw edema in white mice. It shows a significant difference among groups on the reduction of paw size edema over a 6-hour monitoring period, f (4.052), p (0.30) <0.05. This means that the positive control is the most effective followed by the experimental group but not of the negative control. It is further supported by the large effect size (0.252) which reveals that the effect is due to the treatments used.

The significant difference among groups is further supported by the Least Significant Difference. It shows a significant difference in the different treatments used in the reduction of the induced anti-inflammatory effect after treatment over a 6 hour monitoring period, $p < 0.05$.

DISCUSSION

Aloe Vera gel extract produced an acute anti-inflammatory effect. Carrageenan induced inflammatory process is believed to be biphasic. The initial phase seen at the 1st 3 hours is attributed to the release of histamine and Serotonin. The second accelerating phase of swelling is due to the release of prostaglandins, bradykinin, and lysozyme. It has been reported that the second phase of edema is sensitive to both clinically useful steroidal and nonsteroidal anti-inflammatory agents (Bhalsinge et al, 2018). The study of Egesie et al (2011), found out that aqueous extract of the Aloe produced a significant anti-inflammatory effect as indicated by the decrease in paw edema. Aqueous extract of the Aloe reduced the formalin-induced edema significantly at the beginning of 3 hours when compared to the control group. Inhibition of inflammation was observed also in the Formaldehyde induced rat paw edema and in the Histamine-induced edema further supporting the presence of anti-inflammatory substances in the plant.

A novel anti-inflammatory compound called C-glucosyl chromone was isolated from gel extracts of fresh Sabila (Aloe Vera) and it significantly reduced acute inflammation in rats (carrageenan-induced paw edema), but not in chronic inflammation (Bera, 2018). Other aspirin-like compounds are also present in Aloe Vera are responsible for anti-inflammatory and antimicrobial properties. The inhibitory effect of Aloe Vera extract on carrageenan-induced inflammation could also be mediated via the inhibition of cyclooxygenases (COX). Phytochemical analysis of Aloe Vera has revealed the presence of flavonoids, anthraquinones, saponins. Saponins and flavonoids have previously been reported to have anti-inflammatory activities. Purified a high-molecular-weight fraction of Aloe Vera and showed the increased hematological and the hematopoietic activity compared to the gel starting material synthase (also known as cycloxygenase or COX) which catalyzes an early step of prostaglandin synthesis Such compounds may be responsible in part for the described anti-inflammatory activity of Aloe Vera(Subhashis et al, 2014).

Conclusion

The findings of the study showed that Sabila (Aloe Vera) can help in relieving inflammation. In the experiment, the researchers observed that there was a decrease in the size of the swollen paw of the white mice ranging from 0.41 - 0.51 mm to 0.3 mm after six hours with the Sabila (Aloe Vera) extract on the affected area. Aloe Vera showed close to similar results with Hydrocortisone and Petroleum Jelly showing the slight difference, specifically by 0.2mm after six hours Sabila (Aloe Vera) is an effective anti-inflammatory agent as there was a decrease in size observed on the affected part of the White Mice (Mus musculus) Albumin-induced Paw Edema. Aloe Vera decreases the inflammatory phase by its antioxidant properties and promotes the proliferative phase, increasing fibroblast and fibrocytes proliferation and collagen biosynthesis. This research concludes that the effect of natural gel extract of Aloe Vera shows close to similar results with commercialized products.

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Journal Article

Padua FR, Paspe MG. Antinuclear antibody in the rheumatic and non-rheumatic diseases among Filipinos. Acta Med Philippina. 1990;26(2):81-85

One to Six Authors (Commentary, Online)

Krause RM. The origin of plague: old and new. Science. 1992;257:1073-1078

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More than Six Authors

McGlynn EA, M.Asch S, Adams J, et al. The quality of health care delivered to adults in the United States.N Engl J Med. June 26, 2003;348(26):2635-2645.

Authors Representing a Group

Moher D, Schulz KF, Altman D for the CONSORT Group. The CONSORT statement: revised recommendations for improving the quality of reports of parallel-group randomized trials. JAMA. 2011;285(15):1987-1991.

Book

Byrne, DW. Publishing your medical research paper: What they don't teach in medical school. Baltimore: Williams & Wilkins, 1998.

World Wide Web

The key and critical objectives of JAMA. http://jama.ama-assn.org/misc/aboutjama.dtl. Accessed April 4, 2007.

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