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RESEARCH PAPER

THE HISTOLOGICAL EFFECTS OF MICROWAVE OVEN ON BONE DECALCIFICATION USING GOODING AND STEWART FLUID AT VARYING TEMPERATURES AND CONCENTRATIONS

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ABSTRACT

This study evaluates the histological preservation of bone tissue architecture at varying temperatures and concentrations of Gooding and Stewart (GS) decalcifying fluid as the preservation of tissue architecture depends on the quality and velocity of the decalcification processes. In the present study, a decalcification methodology was adopted using household microwave oven to induce the decalcification rate of rabbit compact bone sample for microscopic analysis. Bone biopsy was obtained from rabbit limb and fixed immediately for 24hrs in 10% formalin. Traditional decalcification was carried out at RT (Room Temperature) with 5%-GS as control while microwave oven decalcification was conducted with 5%-GS and 10%-GS at 30^{0C}, 40^{0C} and 50^{0}C respectively. The results showed that 5%-GS and 10%-GS gave excellent histological architecture at 30^{0}C while at 40^{0}C, the histological architectures were comparable to RT decalcification. But at 50^{0}C, bone tissue architecture was completely destroyed being ghost-like in appearance. Of particular interest is the fact that the bone tissue treated with 5%-GS irrespective of the temperature presented excellent tissue architecture than that of 10%-GS. Thus 5%-GS gave the best result in this study.

Key Words: Microwave Oven, Decalcification, Decalcifying fluid, Tissue architecture.

INTRODUCTION

Histologically, traditional decalcification requires long period of time and has been a major deterrent to many types of bone related studies as prolonged exposure to decalcifying fluids with bone tissue causes swelling and hydrolysis to the bone matrix (Keithley *et al.*, 2000 and Pitol *et al.*, 2007). In fact, literature has it that this time lag which is detrimental to bone tissue morphology hinders efficiency in diagnostic results in clinical settings (Madden and Hansen, 1997; Carlton, 1979).

However, the concentration of decalcifying fluid has effect on the tissue as concentration of the acids increases, the faster the time of decalcification but with a concomitant damage to the cell structure (Avwioro, 2010). Also, it has been reported that the acidity/concentrations of demineralization agents affects the stability of proteins, enzyme activities and tissue integrity (Iwasaki *et al.*, 1998).

In addition to, increase in temperature accelerates many chemical reactions including decalcification but a great temperature elevation (50-60^{0}C) is deleterious to tissue morphology (Pitol *et al.*, 2007; Carlton, 1979; Wagenaar et al., 1993). Consequently, several attempts have been made to induce decalcification process to produce minimal effects on tissue morphology after decalcification. This gave rise to histological use of laboratory microwave

(Mayers, 1970) and since then, laboratory microwave have become widely used in laboratory for tissue decalcification. Though, protocols must be carefully optimized to ensure the maintenance of optimal cellular morphology (Marr and Wong, 2009). But laboratory microwave is rarely available and very expensive and because of this, it is not available in most hospitals for laboratory diagnosis. This study therefore, intends to determine the most effective temperature and fluid concentrations for decalcification using Gooding and Stewart fluid at varying temperatures and concentrations using microwave oven.

MATERIALS AND METHODS

Study Location: The study was conducted within Edo state in Ambrose Alli University, histopathology laboratory, Medical laboratory science department and University of Benin Teaching Hospital (UBTH), Edo State.

Sample Population: The total sample of 14 pieces of Rabbit bones was used, 2 pieces for room temperature decalcification and 12 Pieces for the main project work microwave decalcification.

Research Design: This is a study with a pieces of Rabbit compact bone sample, microwave for decalcification at different concentration of Jenkins fluid and Gooding and Stewarts fluid and different range of temperatures. The decalcification conducted at room temperature with 5% Jenkins fluid and 5% Gooding and Stewarts was used as control. The Gooding and Stewarts used at different temperatures and concentrations in microwave were used as the standard while Jenkins fluid was used for the test. The biopsy of the Rabbit limb was collected with container containing 10% formalin and it was fixed for 24hours before decalcification.

Material/ Equipment: Microwave oven, dissecting set, thermometer, small plastic container, universal bottle, test tubes, saw and red litmus paper are used.

Collection of Samples: Biopsy was carried on the Rabbit and their limb was collected and fixed for 24hours. It was cut with saw after 24hours fixation and the pieces of compact bone were fixed after cutting. It was rinsed in distilled water and transferred to 70% alcohol before decalcification. It was properly labeled.

Room Temperature Decalcification Procedure: The pieces of bone tissue were suspended in a universal container containing 5% JK and 5% GS. The decalcifying fluids were changed once daily until decalcification was completed. The bone tissue were rinsed in distilled water and transferred to 70% alcohol before suspending in fresh decalcifying fluid. The chemical method of testing for end point of decalcification was carried out daily to evaluate the progress of decalcification until decalcification is completed. After decalcification was completed, the bone tissues were rinsed in distilled water and transferred to 70% alcohol.

Microwave Decalcification Procedure: The microwave oven is operated at different power and time. The power ranges from p10-p100 and thermometer was used to determine the temperature at each power (p). At power p10, p20, p30, the temperatures were determined to be 30^0C, 40^0C and 50^0C respectively. The decalcification were carried out as follows;

1. Pieces of the bone tissues were suspended in 5% and 10% JK and 5% and 10% GS respectively.
2. It was microwave at 30^0C for 1hour intervals until decalcification is completed.
3. The chemical method of checking end point of decalcification was carried out at 1hour intervals to evaluate the progress of decalcification.
4. The bone tissues were rinsed in distilled water and transferred to 70% alcohol after each 1hour interval of decalcification.
5. The washed bone tissues were suspended in fresh decalcifying fluid at each 1hour intervals.
6. The same processes were carried out with 5% and 10% JK and GS fluids at 40^0C and 50^0C respectively
7. After the decalcification has been completed, the tissues were rinsed in water and transferred to 70% alcohol.

RESULTS

Group A: Control at room temperature with 5% GS: Plate 1 below shows micrographs from bone tissue sections (Compact Bone) 5% GS at room temperature. Plate 1: (5%, GS, RT; H&E x400) presents normal histological features that are distinct with poor staining quality. The features are as indicated in plate 1 with arrows are matured

harvesian system (harvesian canal, blood vessels and osteocyte), young harvesian system, resolving osteon, Volkmann's canal, osteon lamella, lacunae (containing osteocyte or empty in some cases with extracellular fluids), empty lacunae and cement line.

Group B. Microwave oven decalcification at different concentrations and temperatures with Gooding and Stewart fluids: Plate 2 to 7 below showed micrographs from bone tissue section (Compact Bone) decalcified under varied concentrations of decalcifying fluid and temperatures (30^0C, 40^0Cand 50^0C) in microwave oven. Plate 2: (5% GS at 30^0c; H&E x400) presents the best histological features that are distinct with very good staining quality. Plate 3: Compact Bone Section (5% GS at 40^0C) presents normal histological features that are distinct with poor staining quality. Plate 4: Compact Bone Section (5% GS at 50^0C; H&E x400) presents total destruction of the tissue architecture. Plate 5: Compact Bone Section (10% GS at 30^0C) presents normal histological features that are distinct with good staining quality. Plate 6: Compact Bone Section (10% GS at 40^0C; H&E x400) presents normal histological features with good staining quality and distinct histological features. Plate7: Compact Bone Section (10% GS at 50^0C; H&E x400) presents total destruction of the tissue architecture. The observed histological variations were dependent on type of decalcifying fluid, decalcifying fluid concentration, temperature and the rate of decalcification. The histological findings are shown in the plate 1-14 below.

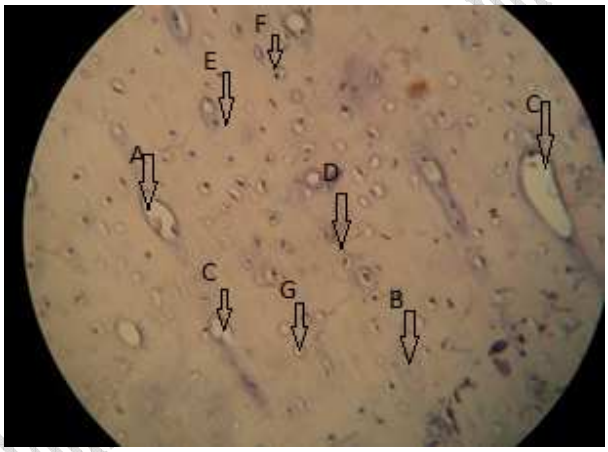

Plate1: Compact Bone Section (5% GS RT; H&E x400) presents normal histological features that are distinct with poor staining quality, showing matured osteon with harvesiancanal, osteocyte and blood vessels **(A)** as well as Young osteon (B), resolving oteon (C), Volkman`s canal (D), osteon lamella (E), lacunae containing osteocyte and empty space occupied with extracellular fluids (F), empty lacunae (G) and cement lines (H). Each of the features are indicated with arrows.

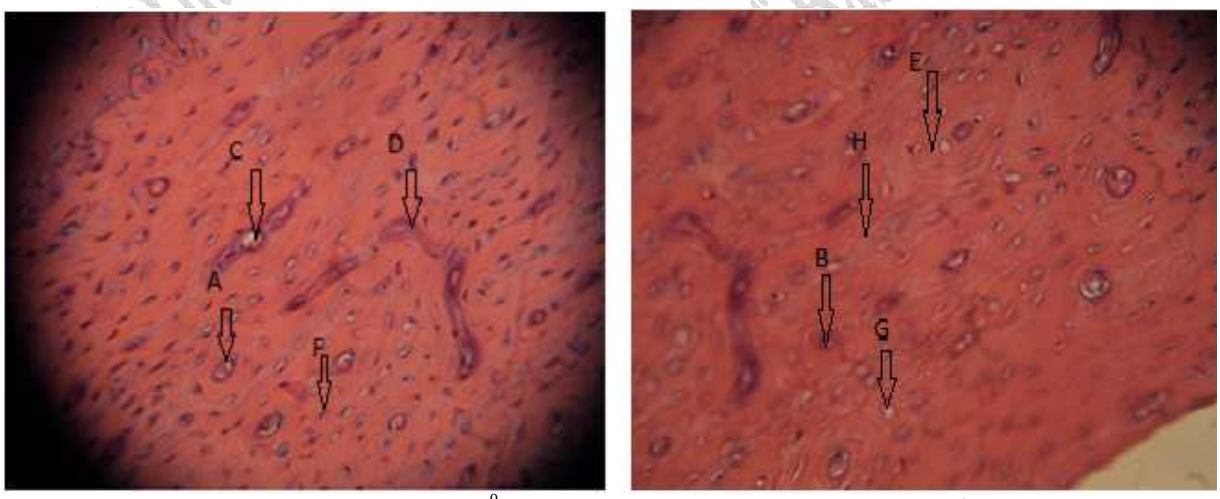

Plate 2: Compact Bone Section (5% GS at 30^0c; H&E x400) presents the best histological features that are distinct with very good staining quality.

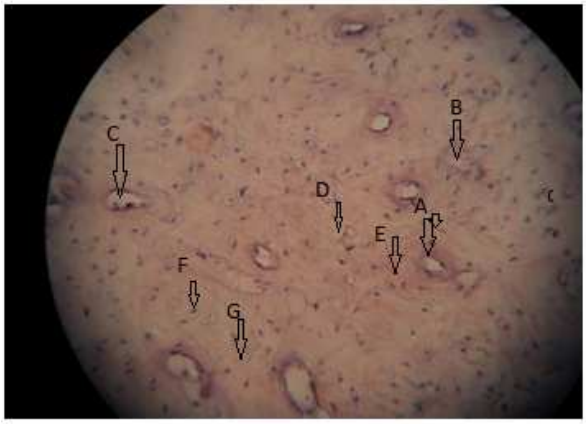

Plate 3: Compact Bone Section (5% GS at 400C) presents normal histological features that are distinct with poor staining quality

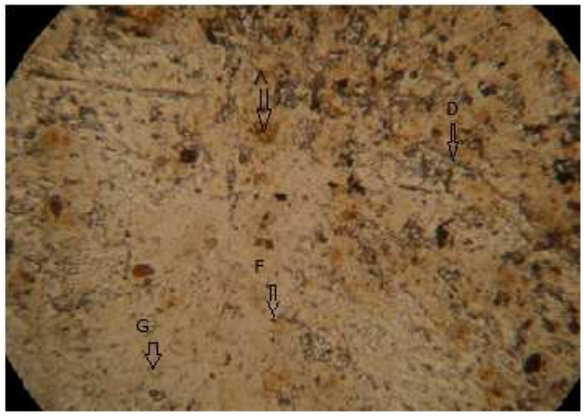

Plate 4: Compact Bone Section (5% GS at 500C; H&E x400) presents total destruction of the tissue architecture

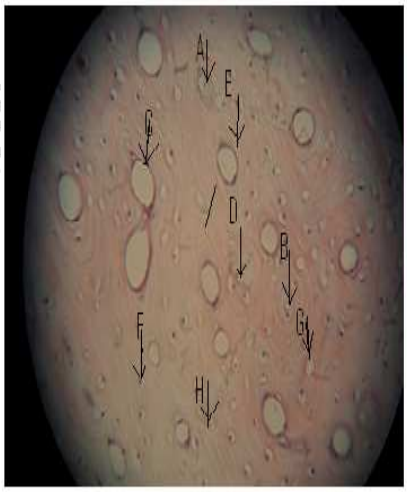

Plate 5:Compact Bone Section (10% GS at 300C) presents normal histological features that are distinct with good staining quality.

Plate 6: Compact Bone Section (10% GS at 400C; H&Ex400) presents normal histological features that aredistinct with good staining quality.

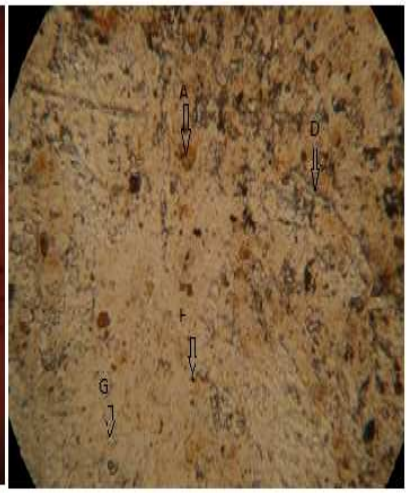

Plate8: Compact Bone Section (10% GS at 500C; H&E x400) presents total destruction of the tissue architecture.

DISCUSSION

The results at room temperature decalcification with 5% GS, are in line with the report of Ochei and Kolhatkar, (2008) and Avwioro (2010), who reported that GS decalcification give normal histological features. The staining quality of 5% GS may be as a result of long time of decalcification and type of decalcifying fluid as reported by Keithley *et al., (*2000) and Pitol *et al.*,(2007) who reported that long time lag is detrimental to bone tissue morphology.

Similarly, the histological finding for 5% GS at 30^0C can be attributed to the moderate increase in temperature and concentration as reported by Guntz and English, (2011).It gave the best result than 5% GS at RT and 5% GS and 10% GS at 40^0C and 50^0C. Also, histological findings for 5% GS at 40^0C can be the as a result of the increase in temperature from 30^0C to 40^0C which is in line with Carleton, (1979) and Wagenaar *et al.*,(1993) but it agrees with Marr and Wong, (2009) who conducted bone decalcification at 37^0C which is within the range of40^0C and obtained

tissue architecture comparable to RT decalcification. While histological findings for 5%-GS at 50^0C is also in line with Carlton, (1979) and Wagenaar *et al.*, (1993).

Furthermore, histological finding for 10% GS at 30^0C can be the result of high concentration which agrees with Iwasaki *et al*., (1998) who reported that the acidity/concentrations of demineralization agents affects the stability of proteins, enzyme activities and tissue integrity and histological findings for 10%GS at 40^0C is in line with the report by Guntz and English, (2011) who also conducted bone decalcification at 40^0C with no adverse effects on structural preservation were noted and nuclear detail was demonstrated to be sharp but disagrees with the report of Iwasaki *et al*.,(1998).While the histological findings for 10%GS at 50^0C is also in line with Carlton,(1979) and Wagenaar *et al.,* (1993).

Meanwhile, the comparative analysis for 5% GS at RT and 30^0C are in lines with the reports of Keithley *et al., (*2000) and Pitol *et al*.,(2007) who reported that long time lag is detrimental to bone tissue morphology as well as Guntz and English, (2011) who reported effects to be due to moderate increase in temperature and concentration. For 5% GS at RT and 40^0C are in lines with the reports of Marr and wong, (2009) who conducted bone decalcification at 37^0C which is within the range of 40^0C and obtained tissue architecture comparable to RT decalcification. Also,for 5% GS at RT and 50^0C is in line with Carlton,(1979) and Wagenaar *et al.,* (1993).

Finally, the comparative analysis for 5% GS at RT and 10% GS at 30^0C are in lines with the reports of Keithley *et al., (*2000),Pitol *et al*.,(2007) and Guntz and English, (2011) as well as Iwasaki *et al*.,(1998). Also, for 5% GS at RT and 10% GS at 40^0C is in lines with the reports of Keithley *et al. (*2000) and Pitol *et al* (2007) and Marr and wong, (2009).While that for 5% GS at RT and 10% GS at 50^0C was in line with Carleton (1979) and Wagenaar *et al.,* (1993) as well as Iwasaki *et al*.,(1998).

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AUTHOR(S) CONTRIBUTION

All authors (Eloka C.C.V., Okon A.C., Eneasato A.P., Okoro C.J., Ekoh, S.N., Ezeah, G.A.C .) actively took part in this study and in the presentation of this article.