CHAPTER FOUR: RESULTS

4.1 pH of Qat Extract

The qat extract was replaced every other day. The pH of the fresh prepared extract was measured and is presented in Table 4.1. The mean pH of 10% qat extract was 5.31± (0.009) and 5.33± (0.013) for 20% qat extract.

Table 4.1 pH of 10% and 20% qat

Day	1	3	5	7	9	11	13	15	17	19	21	23	25	27
pH 10% qat	5.3	5.32	5.31	5.3	5.3	5.32	5.31	5.31	5.32	5.3	5.32	5.3	5.3	5.31
pH 20% qat	5.34	5.32	5.34	5.34	5.34	5.33	5.34	5.34	5.34	5.3	5.31	5.34	5.34	5.34

4.2 Gross Examination of Specimens

Under stereomicroscope at 10x magnification, the smooth enamel surfaces and the enamel surrounding restoration in the acid gel group appeared rough and chalky white as shown in Figure 4.1. However the area below CEJ of most specimens appeared brownish. Similarly, rough enamel surface was also observed in specimens immersed in both qat extracts. However, the enamel was stained dark brown as shown in Figure 4.2.

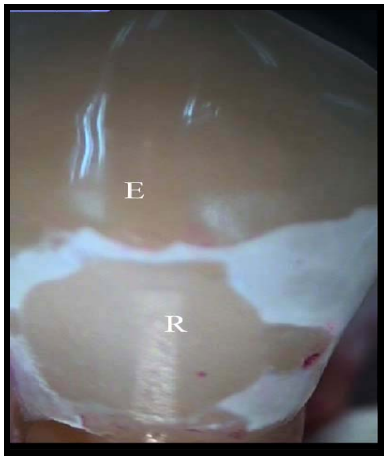


Figure 4.1 Chalky appearances of enamel around restoration (acid gel group) after 4 weeks of immersion (E: Enamel, R: Restoration)

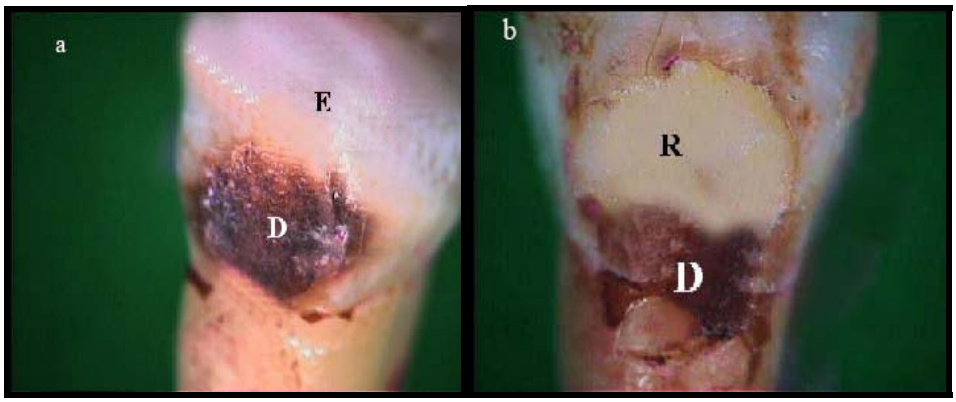


Figure 4.2 Discolorations of enamel after immersion in 10% qat extract (a) and around restoration (b) after 4 weeks of immersion.
(E: Enamel, D: Discolored surface, R: Restoration)

4.3 Smooth Enamel Surface

4.3.1 Histopathology of demineralized area

Under polarized light microscopy at 20x magnification, smooth enamel surface of specimens immersed in acid gel showed caries-like lesion and the outer surface appeared slightly irregular. The body lesion showed positive birefringence and the demineralized area can be described as "waterfall-like". The body lesion could be clearly observed because of changes in birefringence as shown in Figure 4.3.

Smooth enamel surface of specimens immersed in 10% qat and 20% qat extract showed caries-like lesion with irregular enamel surfaces. However the body lesion appeared to be more uniform as shown in Figure 4.4 and 4.5. None of specimens immersed in qat extract showed advanced lesion up to the enamel–dentine junction. Whilst, 60% of specimens immersed in acid gel showed lesions reaching to the enamel-dentine junction. Specimens in all groups lacked negative birefringence at the surface zone causing the surface layer to be seen as continuous with the subjacent body of the lesion which exhibits positive birefringence as shown in Figures 4.3, 4.4 and 4.5.

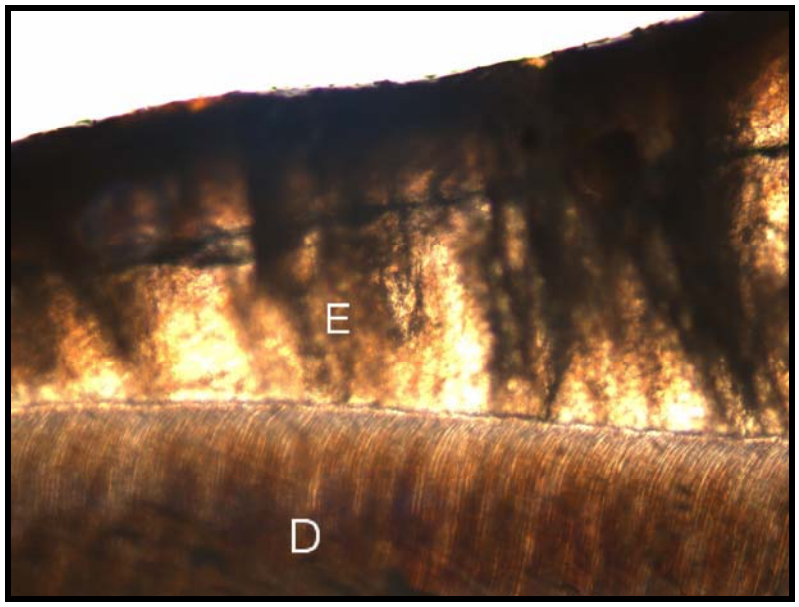


Figure 4.3 Smooth enamel surface lesion in acid gel group at 20X magnification after 4 weeks of immersion. (E: Enamel, D: Dentine)

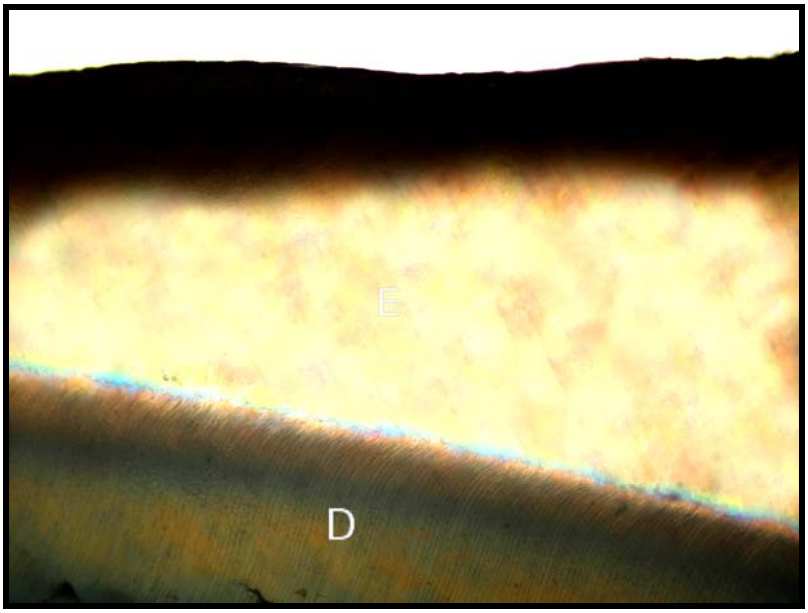


Figure 4.4 Smooth enamel surface lesion in 10% qat extract group at 20X magnification after 4 weeks of immersion.
(E: Enamel, D: Dentine)

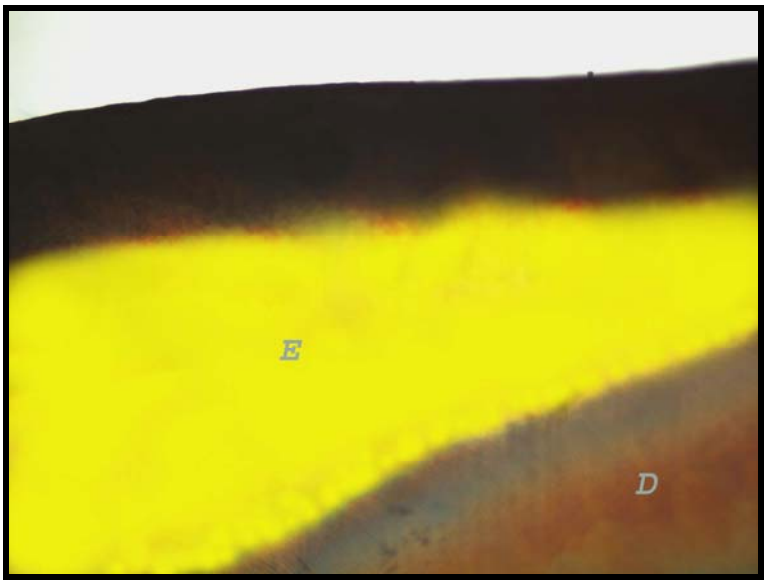


Figure 4.5 Smooth enamel surface lesion in 20% qat extract at 20X magnification after 4 weeks of immersion(E: Enamel, D: Dentine)

All measurements were carried out under polarized light microscopy at 20X magnification using image analyzer soft ware linked to a microscope.

4.3.2 Depth of smooth enamel surface lesion

The mean enamel lesion depth for each group is presented in Figure 4.6. The mean enamel surface lesion depth was 311.25 μm ± 71.07 for acid gel, 146.45 μm ± 33.76 for 10% qat and 153.89 μm ± 44.68 for 20% qat extract.

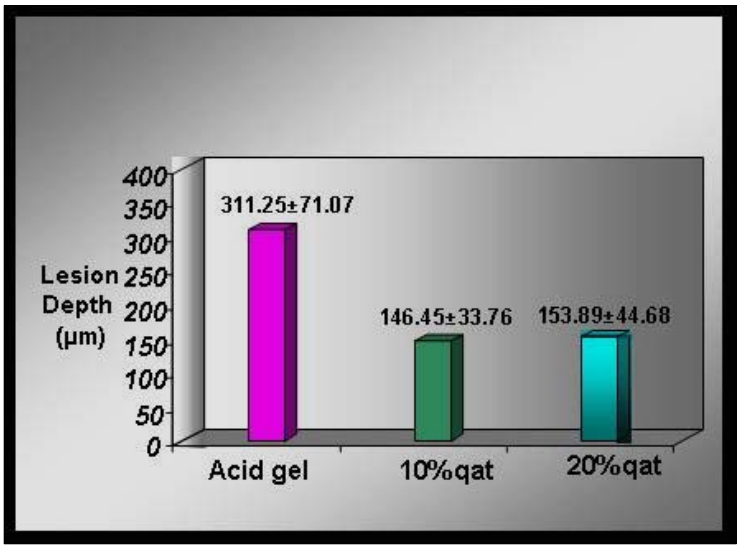


Figure 4.6 Mean depth of smooth enamel surface lesion

4.3.2.1 Intra-examiner reliability

The intra-class correlation coefficient obtained for the acid gel group was 0.97. The high value indicate that the measurements carried out by the investigator of the lesion was consistent and, therefore, reliable.

4.3.3 Statistical analysis

4.3.3.1 Preliminary Analysis

Assumption of normality was checked using the SPSS version 12 Explore procedure. The results of the analysis indicated that the distributions for the three groups approximate the normal distribution as the skewness and kurtosis values for the groups are between -1 and +1 (Table 4.2). The 10% qat extract group, however, shows a slightly high kurtosis value (-1.038). Nonetheless, this value is still within acceptable range -2 and +2 as described by Tabachnick & Fidell, (2001).

Table 4.2 Skewness and Kurtosis Values of enamel lesion depth

	acid gel	10% qat extract	20% qat extract
Skewness	**-.451**	**-.035**	**-.315**
Kurtosis	**-.433**	**-1.038**	**-.527**

Boxplot in Figure 4.7 also indicated the absence of outliers in each of the three groups. However, the positions of the median lines for the acid gel and 20% qat extract groups suggest slight negative skewness in the distributions. This is reflected in the values presented in Table 4.2. Additionally, based on the box height (interquartile ranges); it appears that the acid gel group show greater variation within groups than the other two groups. This suggests a potential problem with homogeneity of variance. However, as sample sizes are equal, the robustness of the statistical test is expected (Tabachnick & Fidell, 2001).

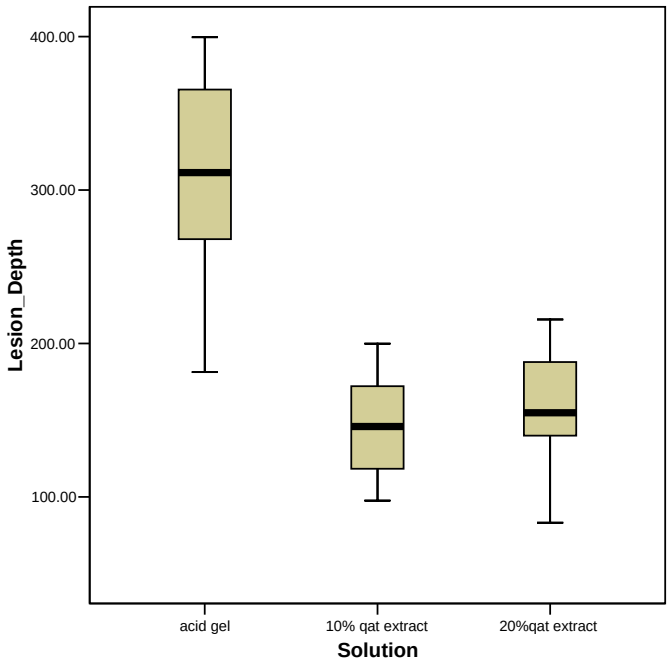


Figure 4.7 Boxplots of Outer Lesion Depth for the Three Groups

4.3.3.2 One-way ANOVA

The sample size, variability, and the mean values of outer lesion depth for the three groups are displayed in Figure 4.6. The results of the statistical analysis using the one-way ANOVA are presented in Table 4.3. The large effect size as indicated by the high value of eta square (η^2), .70 which demonstrate the strength of the relationship between the demineralizing agent or solution type and the extent of enamel demineralization. The ANOVA was significant, $F(2, 27) = 31.71$, $p < .005$. This indicates that the means for the three groups are significantly different.

Table 4.3 One-way Between-subjects ANOVA

Tests of Between-Subjects Effects

Dependent Variable: Lesion_Depth

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power[a]
Corrected Model	173106.554[b]	2	86553.277	31.711	.000	.701	63.423	1.000
Intercept	1247146.196	1	1247146.196	456.930	.000	.944	456.930	1.000
Solution	173106.554	2	86553.277	31.711	.000	.701	63.423	1.000
Error	73693.928	27	2729.405					
Total	1493946.679	30						
Corrected Total	246800.482	29						

a. Computed using alpha = .05

b. R Squared = .701 (Adjusted R Squared = .679)

A post-hoc analysis was conducted for pair-wise comparisons using Dunnett t-tests for the following reasons:

i) Comparison of the test groups (10% qat extract and Qat 20%) against the control group (acid gel).

ii) Assumption of homogeneity of variance had been met as illustrated in Table 4.4. Significant differences were found between both the test groups (10% and 20% qat extract) and the control group (acid gel). The means of enamel lesion depth for 10% and 20% qat extract groups are significantly lower than the acid gel group at $p<.05$ (Table 4.5).

Table 4.4 Test of Homogeneity of Variances

Levene Statistic	df1	Df2	Sig.
2.401	2	27	.110

Another post hoc test was conducted using Tukey HSD to investigate the effect of two different qat concentrations on the extent of enamel demineralization. There was no significant difference between the 10% and 20% qat extract groups (p> .05) as shown in Figure 4.5

Table 4.5 Tukey HSD and Dunnett t-tests

Multiple Comparisons

Dependent Variable: Lesion_Depth

	(I) Solution	(J) Solution	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Tukey HSD	acid gel	10% qat extract	164.69050*	23.36410	.000	106.7611	222.6199
		20%qat extract	157.33700*	23.36410	.000	99.4076	215.2664
	10% qat extract	acid gel	-164.69050*	23.36410	.000	-222.6199	-106.7611
		20%qat extract	-7.35350	23.36410	.947	-65.2829	50.5759
	20%qat extract	acid gel	-157.33700*	23.36410	.000	-215.2664	-99.4076
		10% qat extract	7.35350	23.36410	.947	-50.5759	65.2829
Dunnett t (2-sided)[a]	10% qat extract	acid gel	-164.69050*	23.36410	.000	-219.2086	-110.1724
	20%qat extract	acid gel	-157.33700*	23.36410	.000	-211.8551	-102.8189

*. The mean difference is significant at the .05 level.

a. Dunnett t-tests treat one group as a control, and compare all other groups against it.

4.4 Restoration Interface

4.4.1 Histopathology of enamel demineralization at restoration interface

Examination of enamel around restoration was carried out under polarized light microscope at 20X magnification for the coronal and cervical area of restoration and this was divided into:-

a) Outer surface lesion.

b) Wall lesion (enamel adjacent restoration interface).

Caries-like lesion was observed in all specimens immersed in acid gel. This can be clearly seen due to change of birefringence as shown in Figure 4.8. The shape of the outer lesion and its relationship to the wall lesion was influenced by the direction of the enamel prisms. The outer surface lesion at coronal area follows the direction of the enamel prisms i.e. toward the cavity wall. At cervical area the lesion also follows the direction of the enamel prisms (Fig 4.9).

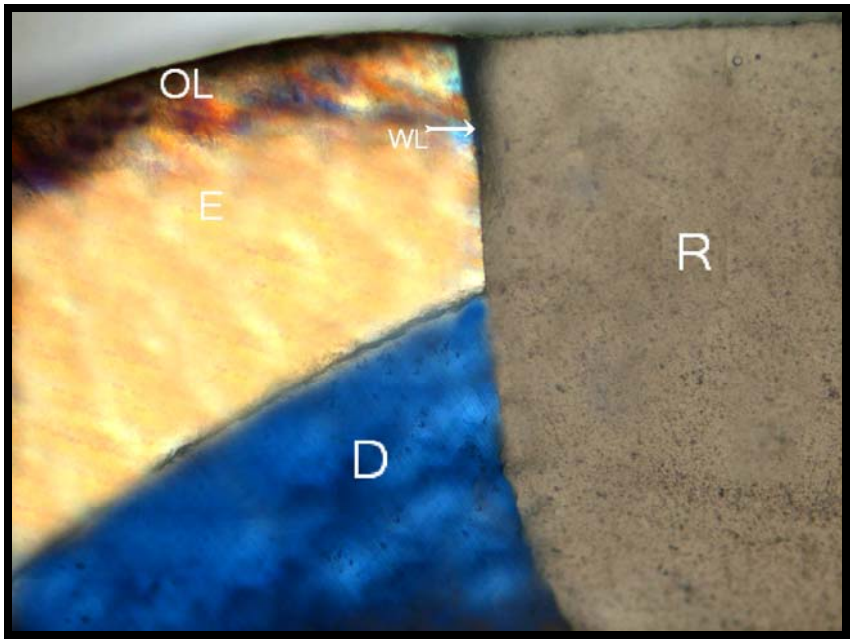


Figure 4.8 Caries-like lesion at restoration interface at the coronal area (acid gel group) at 20X magnification after 4 weeks of immersion. Lesion consists of an outer lesion (OL) and wall lesion (WL). (R: Restoration, E: Enamel, D: Dentine

All specimens immersed in acid gel showed wall lesion at coronal and cervical area of restoration. The wall lesion at coronal area appeared as dark line at cavity wall. This line has been described as ribbon-like extension by Gilmour & Edmunds, (1998), as shown in Figure 4.9. However, the wall lesion in 30% of specimens appeared as downturn of outer lesion (Figure 4.9). Similarly, the shape of wall lesion at cervical area also appeared as, ribbon-like extension as shown in Figure 4.10. Opening V

shaped notch at enamel margin was observed in three specimens immersed in acid gel as shown in Figure 4.11

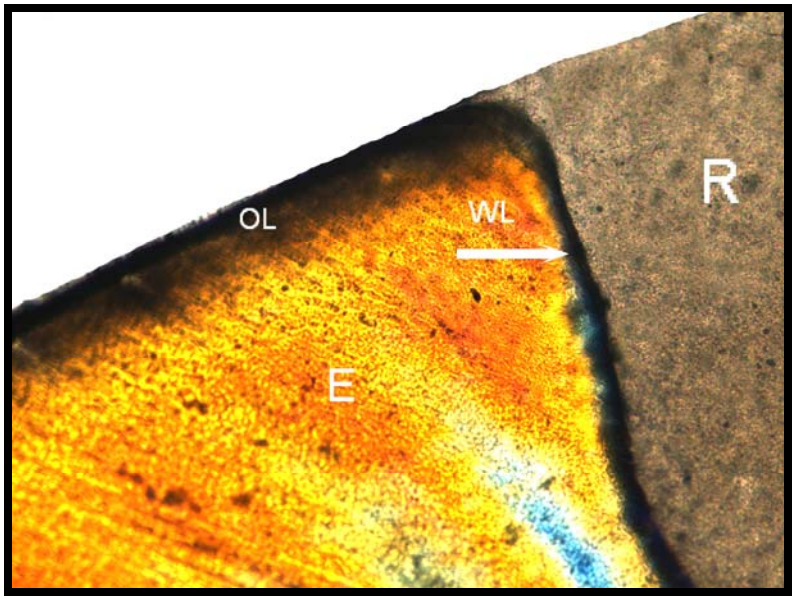


Figure 4.9 Wall lesion appeared as ribbon-like extension (indicated by arrow) at coronal area in acid gel group at 40X magnification after 4 weeks of immersion. (OL: Outer lesion, WL: Wall lesion, E: Enamel, D: Dentine, R: Restoration)

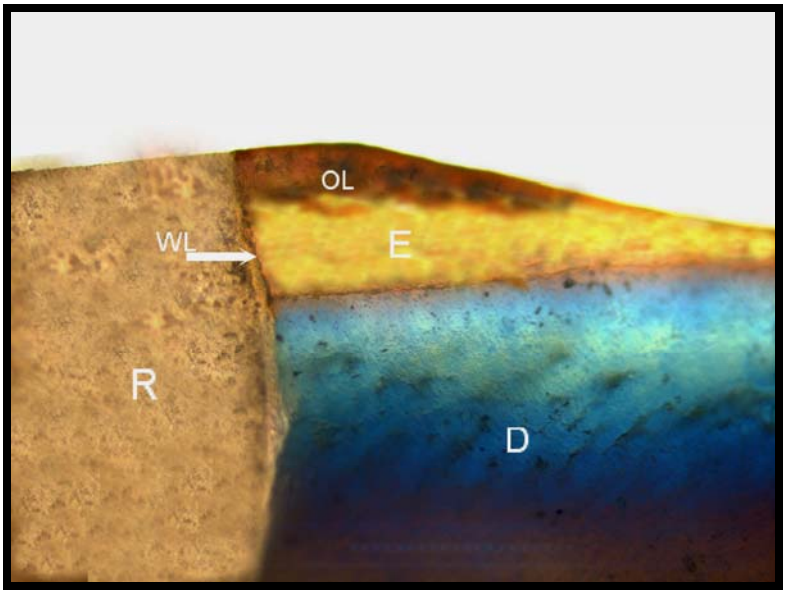


Figure 4.10 Wall lesion appeared as ribbon-like extension (indicated by arrow) at cervical area due to acid gel at 20X magnification after 4 weeks of immersion. (OL: Outer lesion, WL: Wall lesion, E: Enamel, D: Dentine, R: Restoration)

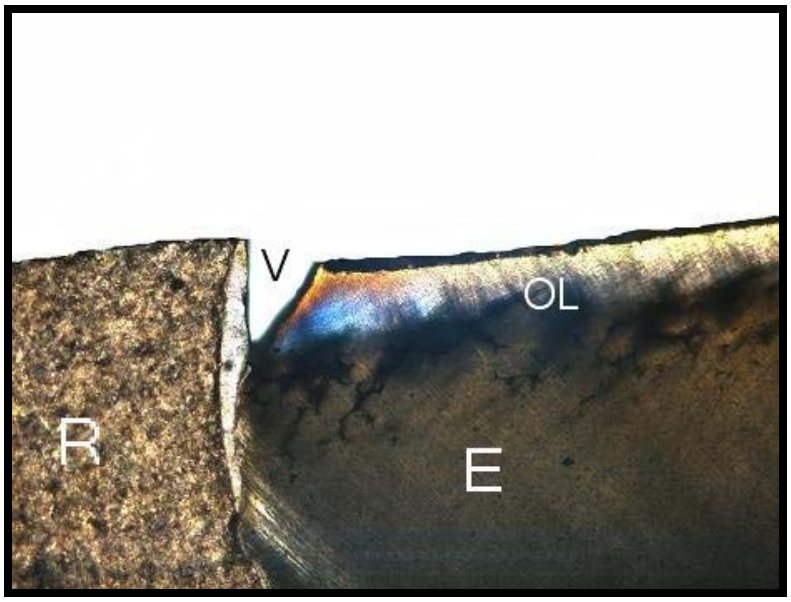


Figure 4.11 "V" shape notch at cervical enamel margin of acid gel group at 40X magnification after 4 weeks of immersion.
(OL: outer lesion, E: Enamel, R: Restoration)

The specimens immersed in both 10% qat extract and 20% qat extract exhibited similar feature of caries-like lesion as shown in Figure 4.12. The outer lesion appeared to be directed towards the cavity wall. Wall lesion in most of specimens appeared as ribbon-like extension as shown in Figure 4.13. 70% of specimens immersed in both 10% and 20% qat extract showed wall lesion. However, no wall lesion in some specimens was clearly seen in 20% qat extract as shown in Figure 4.14. None of specimens immersed in 10% and 20% qat extract showed" V" shape notch at enamel margin.

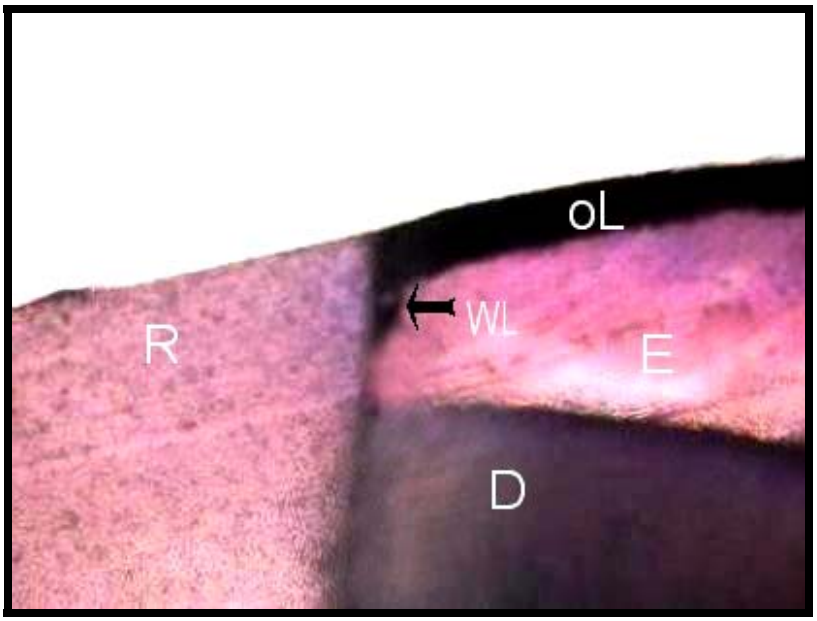


Figure 4.12 Caries-like lesion at restoration interface at coronal area (10% qat extract group) at 20x magnification after 4 weeks of immersion. Lesion consists of an outer lesion (OL) and wall lesion (WL). R: Restoration E: Enamel, D: Dentine)

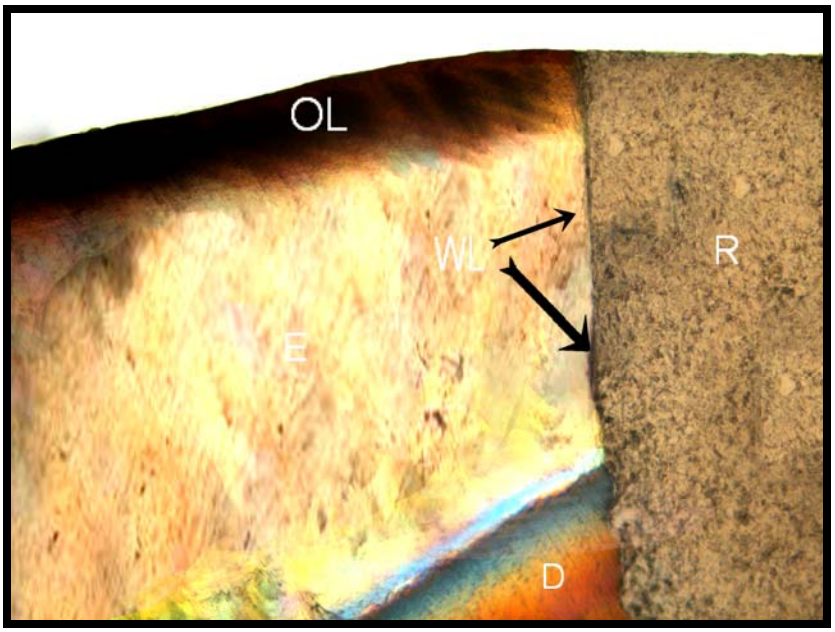


Figure 4.13 Wall lesion (WL) appeared as ribbon-like extension (indicated by arrows) at coronal part of 10%qat extract groups at 20X magnification after 4 weeks of immersion. (OL: outer lesion, E: Enamel, D: Dentine, R: Restoration)

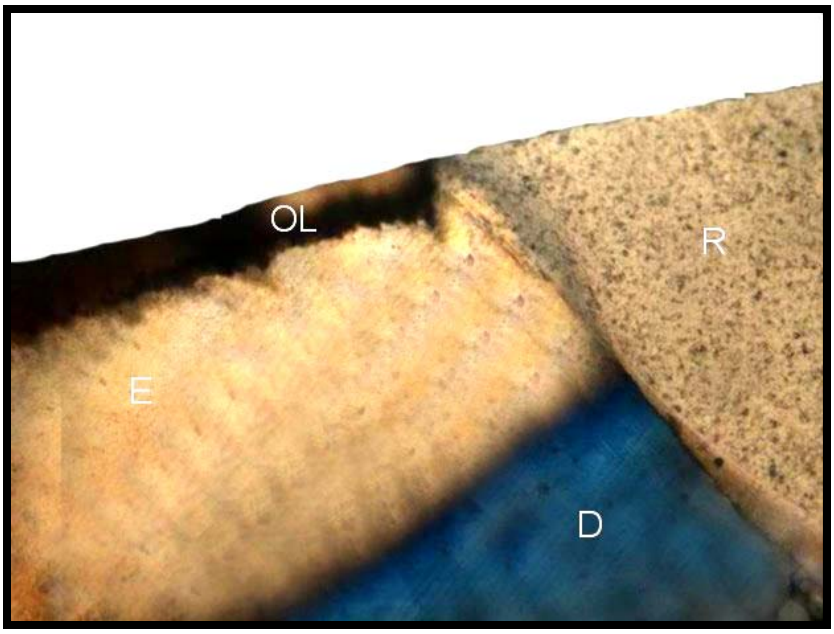


Figure 4.14 No wall lesion observed in 20% qat extract group at 20X magnification after 4 weeks of immersion. (OL: outer lesion, E: Enamel, D: Dentine, R: Restoration).

4.4.2 Depth of the lesion at restoration interface

4.4.2.1 Outer surface lesion depth at coronal and cervical area

The mean lesion depth for each specimen for outer lesion at coronal and cervical area of restoration is presented in Figure 4.15. The mean coronal enamel outer lesion depth was 256.28 µm ± 142.77 for acid gel, 77.24 µm ± 47.46 for 10% qat extract and 104.87 µm ± 58.43 for 20% qat extract. The mean cervical outer lesion depth was 253.93 µm ± 98.07 for acid gel, 99.70 µm ± 72.70 for 10% qat extract and 116.81 µm ± 58.20 for 20% qat extract.

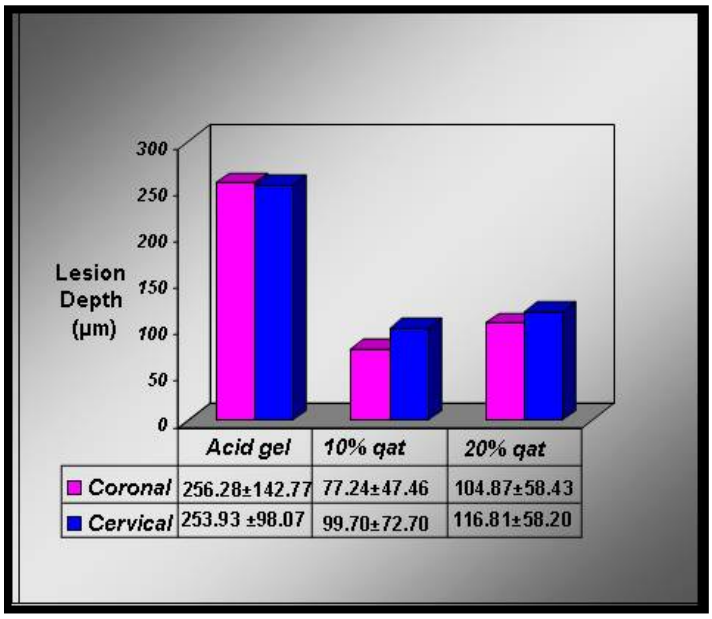


Figure 4.15 Mean outer lesion depth at coronal and cervical area of restoration

4.4.2.2 Wall lesion depth at coronal and cervical area

The mean wall lesions depth at coronal and cervical area of restoration interface is presented in Figure 4.16. The mean coronal wall lesion depth was 193.61µm ± 120.50 for acid gel, 116.42 µm ± 117.50 for 10% qat extract and 72.42 µm ± 65.61 for 20% qat extract. The mean wall lesion at cervical area was 251.07 µm ± 102.56 for acid gel, 150.11 µm ± 106.25 for 10% qat extract and 111.58 µm ± 81.68 for 20% qat extract.

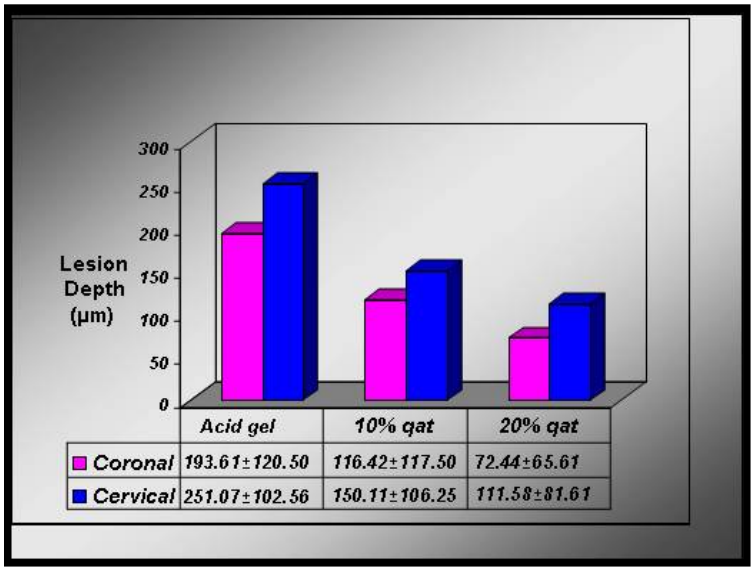


Figure 4.16 Mean wall lesion depth at coronal and cervical area of restoration

4.4.2.3 Intra-examiner reliability

The intra-class correlation coefficients obtained for the specimens in acid gel group are presented in Table 4.6. The high alpha values indicate that the measurement of the demineralization at restoration interface as measured by the outer and the wall lesions was consistent and, therefore, reliable.

Table 4.6 Intra-class correlation coefficient for Intraexaminer reliability

Coronal Outer Lesion	Coronal Wall Lesion	Cervical Outer Lesion	Cervical Wall Lesion
.84	.99	.94	.99

4.4.3 Statistical analysis

4.4.3.1 Preliminary analysis

Initial data exploration showed acceptable skewness and kurtosis values between -2 and +2 with the exception of the coronal outer lesion for 20% qat extract group, kurtosis value of 2.755 (Appendix C, Table1). Boxplots indicated the presence of several extreme value and outliers as shown in, Figure 1, 2, and 3, Appendix C, except for cervical wall lesion as illustrated in Figure 4, Appendix C. As these outliers and extreme values are likely to affect the results of the MANOVA, they were subsequently removed from the data set. Tables 4.7 and 4.8 present the means and standard deviations for each level of the demineralizing agent (IV) on the extent of demineralization at four different sites (DV) before and after the removal of the outlier and extreme values.

Table 4.7 Means and Standard Deviations before Removal of Outliers & Extreme Values

DV					
IV		**Coronal outer lesion**	**Coronal wall lesion**	**Cervical outer lesion**	**Cervical wall lesion**
Acid gel	**Mean**	**256.28**	**193.61**	**253.93**	**251.07**
	SD	**142.78**	**120.50**	**98.08**	**102.56**
10% qat extract	**Mean**	**77.24**	**116.43**	**99.71**	**150.12**
	SD	**47.47**	**117.50**	**72.70**	**106.25**
20% qat extract	**Mean**	**104.88**	**72.42**	**116.81**	**111.58**
	SD	**58.44**	**65.62**	**58.20**	**81.68**

Table 4.8 Means and Standard Deviations after Removal of Outliers & Extreme Values

DV					
IV		**Coronal outer lesion**	**Coronal wall lesion**	**Cervical outer lesion**	**Cervical wall lesion**
Acid gel	**Mean**	**256.28**	**193.61**	**253.93**	**251.07**
	SD	**142.78**	**120.50**	**98.08**	**102.56**
10% qat extract	**Mean**	**57.24**	**116.69**	**66.68**	**157.25**
	SD	**24.64**	**118.10**	**21.93**	**100.01**
20% qat extract	**Mean**	**89.71**	**56.31**	**102.19**	**93.53**
	SD	**35.39**	**43.85**	**37.43**	**61.98**

Table 4.9 gives the skewness and kurtosis values after removal of outliers and extreme values. From the table the only high value is the kurtosis value for coronal wall lesion for 10% qat extract (-2.032).

Table 4.9 Skewness and Kurtosis Values of the Dependent Variables for the Different Levels of the IV after removal the outliers

DV					
IV		**Coronal outer lesion**	**Coronal wall lesion**	**Cervical outer lesion**	**Cervical wall lesion**
Acid gel	**Skewness**	**.294**	**-.157**	**.373**	**.095**
	Kurtosis	**-.807**	**-.113**	**-.316**	**-.981**
10% qat extract	**Skewness**	**.973**	**.253**	**-.211**	**-.565**
	Kurtosis	**-.227**	**-2.032**	**-.813**	**-1.543**
20% qat extract	**Skewness**	**1.225**	**.318**	**.880**	**.739**
	Kurtosis	**1.865**	**-.422**	**.036**	**.269**

The assumption of linearity was examined using scatterplots and linear relationships between all pairs of the DVs and is shown in (Figure 4.17).

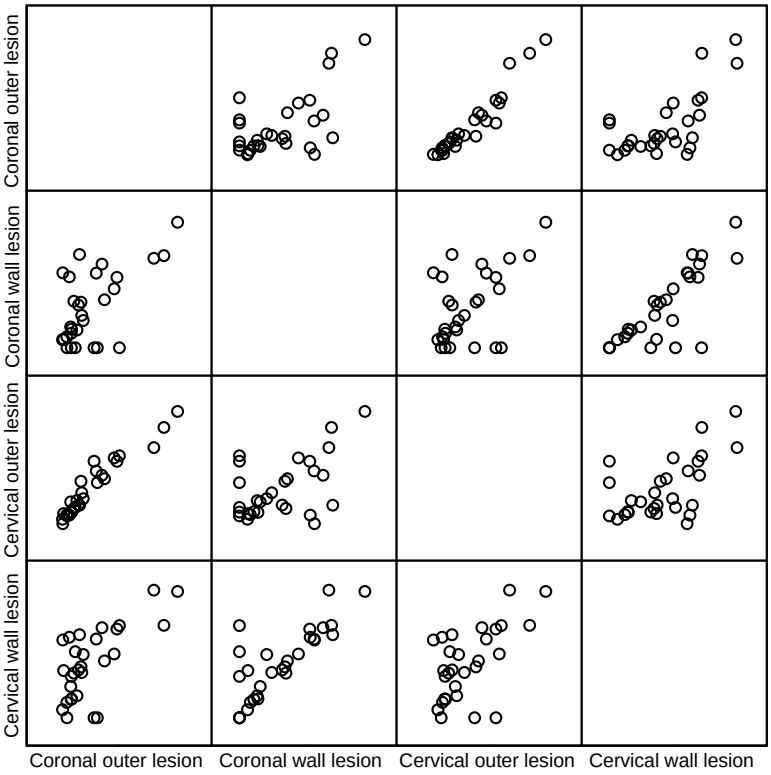


Figure 4.17 Scatterplots of All Pairs of DVs

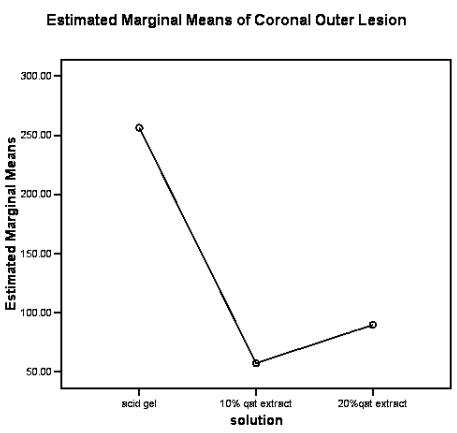
The multivariate test (Box's Test) revealed that the homogeneity of variance and covariance matrices cannot be supported (Appendix C, Table 2). However, as the sample sizes are almost equal, robustness of the significant tests is expected. Furthermore, the option of using Pillai's criterion, which is said to be more robust than the other statistics (i.e., Wilks' Lambda, Hotelling's Trace and Roy's Criterion), to evaluate multivariate significance will ensure robustness of the significance test despite the violation of homogeneity of variance-covariance matrices.

4.4.3.2 One-way Between-Subjects MANOVA

A one-way between-subjects MANOVA was performed on 4 dependent variables: coronal outer lesion, coronal wall lesion, cervical outer lesion and cervical wall lesion. With the use of Pillai's Trace criterion, the combined DVs were found to be

significantly affected by the different levels of the IV, F (8,42) = 4.799, p< .005(Appendix C, Table 3). The partial eta squared ($\eta^2 = .48$) indicates a quite strong relationship between IV and the combined DVs.

Figures 4.18 to 4.21 give the graphic presentation of the means of the three groups for each of the DVs.



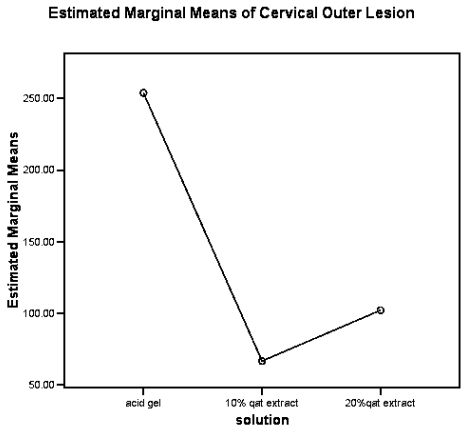
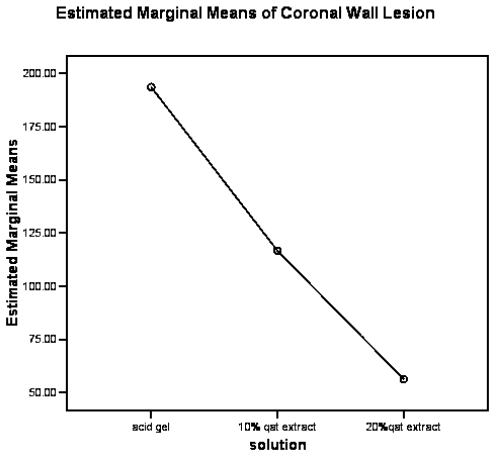


Figure 4.18 Means of coronal outer lesion Figure 4.19 Means of cervical outer lesion



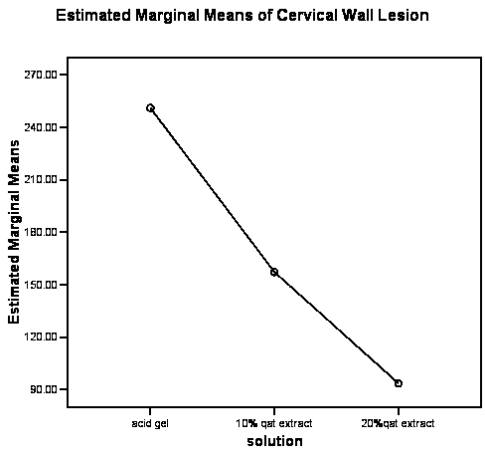


Figure 4.20 Means of coronal wall lesion Figure 4.21 Means of cervical wall lesion

Analyses of variances (ANOVA) on each dependent variable were subsequently conducted as follow-up tests to the MANOVA. The univariate ANOVAs on each of the dependent variables yielded significant results, $p < .05$. The details of the results are presented in Table 4.10.

Post hoc analyses to the univariate ANOVAs were then conducted using the following statistical tests: Dunnett T3 and Dunnett t (2-sided). These tests were chosen for several reasons. Since this research involves comparisons against a control group, the Dunnett t tests was considered an appropriate post hoc test. However, as it is of interest to examine whether significant differences exist between the 10% and 20% qat Extract, another post hoc test (Dunnett T3) was chosen. This test was also chosen due to the significant results of the Levene's test for three of four of the DVs (Appendix C, Table 4). It was essential that a test that does not require the assumption of homogeneity of variance be used.

Table 4.10 Univariate Tests on Each of the DVs

Source	Dependent Variable	Type III Sum of Squares	df	F	Sig.	Partial Eta Squared	Observed Power(a)
Solution	Coronal Outer Lesion	199078.301	2	12.909	.000	.529	.993
	Coronal Wall Lesion	85218.210	2	4.276	.026	.271	.687
	Cervical Outer Lesion	172330.742	2	21.643	.000	.653	1.000
	Cervical Wall Lesion	112933.793	2	7.024	.004	.379	.891

The pairwise multiple comparisons indicated that there is significant differences between both qat extract groups(10% & 20%) and acid gel group for coronal outer and cervical outer restoration interfaces ($p < 0.05$) as shown in Table 4.11 and SPSS output in Appendix C, Table 6. Although the acid gel group showed greater outer lesion depth at coronal and cervical part of restoration, no significant difference was found between

10% and 20% qat extract groups. A significant difference between acid gel and 20% qat extract was found for the coronal wall and cervical wall at restoration interfaces. However, there are no significant differences between the 10% qat extract and acid gel and between 10% and 20% qat extract ($p < .05$).

Table 4.11 Summarized comparison between groups using Dunett T3

	group / group	**10% qat extract**	**20% qat extract**
Coronal outer lesion	**Acid gel**	**.008**	**.022**
	10% qat		**.120**
Coronal wall lesion	**Acid gel**	**.480**	**.026**
	10% qat		**.473**
Cervical outer lesion	**Acid gel**	**.001**	**.004**
	10% qat		**.087**
Cervical wall lesion	**Acid gel**	**.203**	**.005**
	10% qat		**.361**

Significant difference at p<.05

A 3 x 2 ANOVA was conducted to evaluate the effects of solution type (acid gel, 10% qat extract and 20% qat extract) and location of restoration (coronal wall and cervical wall) on demineralization as measured by wall lesion depth. The ANOVA indicated no significant interaction between demineralizing agent/solution type and location of lesion, $F(2, 52) = 0.077$, $p = .926$, $\eta^2 = .003$, but significant main effect for solution type, $F(2, 52) = 9.861$, $p < .001$, $\eta 2 = .275$. The location main effect, on the other hand, was not significant, $F(1, 52) = 3.439$, $p = .069$, $\eta^2 = .062$. This suggests that demineralization of the enamel is significantly different for demineralizing agent but is not significantly different for location of restoration interface (Table 4.13). Figure 4.22 shows the mean of lesion depth at the two margin location.

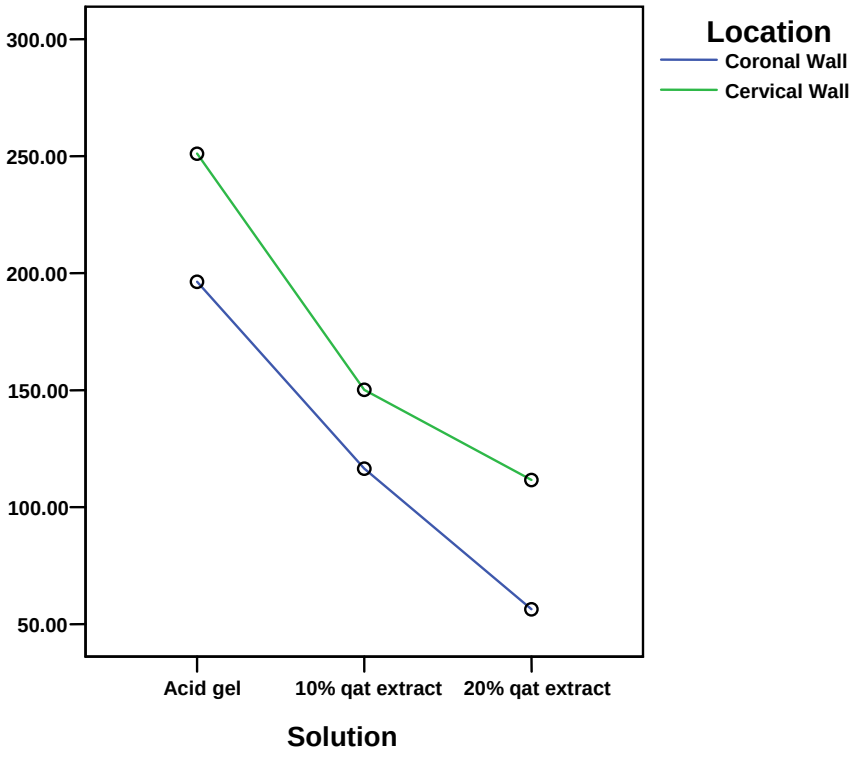


Figure 4.22 Estimated Marginal Means of Coronal and cervical Wall Lesion

Table 4.12 Results of the Two-way ANOVA

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	217773.141(b)	5	43554.628	4.509	.002	.302
Intercept	1249601.773	1	1249601.773	129.375	.000	.713
Solution	190492.615	2	95246.307	9.861	.000	.275
Location	33213.053	1	33213.053	3.439	.069	.062
solution * Location	1491.385	2	745.693	.077	.926	.003
Error	502255.552	52	9658.761			
Total	1968765.554	58				
Corrected Total	720028.693	57				

a Computed using alpha = .05
b R Squared = .302 (Adjusted R Squared = .235)

Given the significant results of the main effect for demineralizing agent/solution, follow-up analyses of all pairwise comparisons among the three types of solution were conducted. A post hoc multiple comparison Dunnett T3 test was chosen as equal variance of all group can not be assumed base on the levens test (Appendix C, Table5). The results of this analysis indicate that the two qat extracts caused significantly less demineralization of the enamel when compared to acid gel. The results also indicate that there is no significant difference between the two qat extract groups (Table 4.13) and SPSS output in Appendix C, Table 7.

Table 4.13 Multiple Pairwise Comparison Using the Dunnett T3 Test

	Coronal wall lesion			
Cervical wall lesion	**Solution**	**Acid gel**	**10% qat**	**20% qat**
	Acid gel		**.046**	**.000**
	10% qat	**.046**		**.301**
	20% qat	**.000**	**.301**	

Significant difference (P<0.05)