RESULTS

GROSS FINDINGS:

Colour:

The colour of tissues was examined and predominant primary colour of tissue was noted.

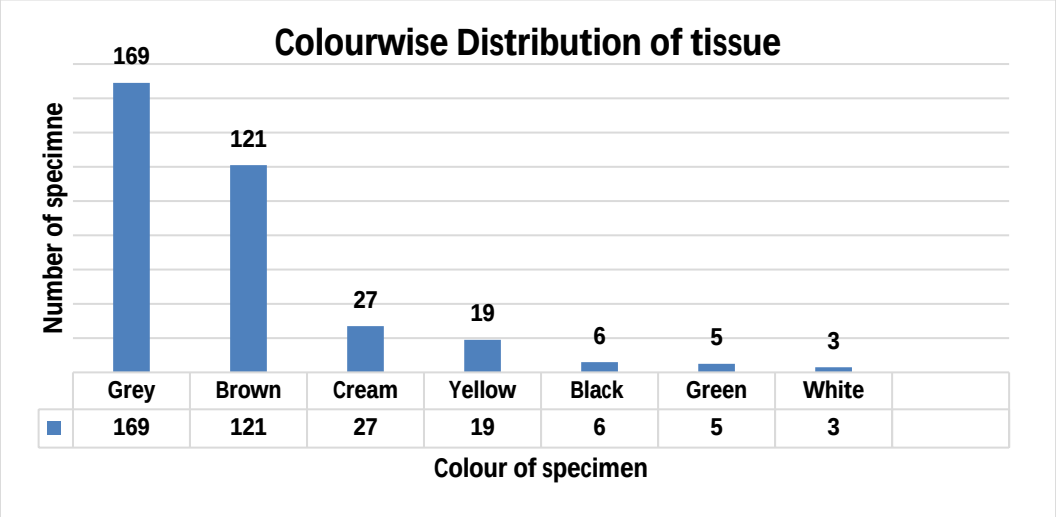

Figure 5.1 Colourwise distribution of tissues

Consistency:

Consistency of tissues was recorded while grossing of specimen and subsequent sections were taken. If the tissues were hard, they were not included in the study.

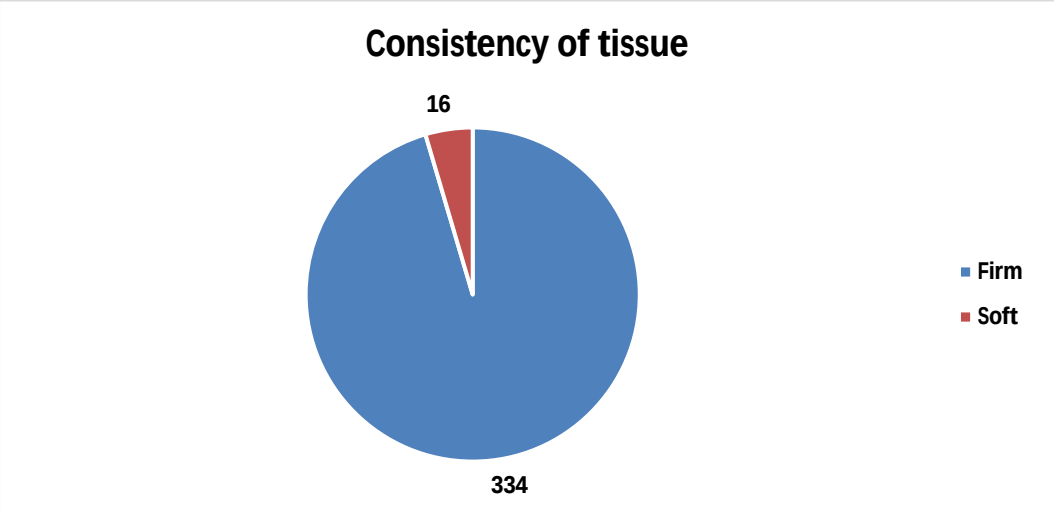

Figure 5.2 Consistency wise Distribution of tissue

Table 5.1 Average dimensions of tissue before processing and after processing:

				Routine method			**Microwave method**		
	Before processing			**After processing**			**After processing**		
	Length	Breadth	Width	Length	Breadth	Width	Length	Breadth	Width
Mean (cm)	1.34	1.15	0.38	1.23	1.04	0.29	1.21	1.03	0.27

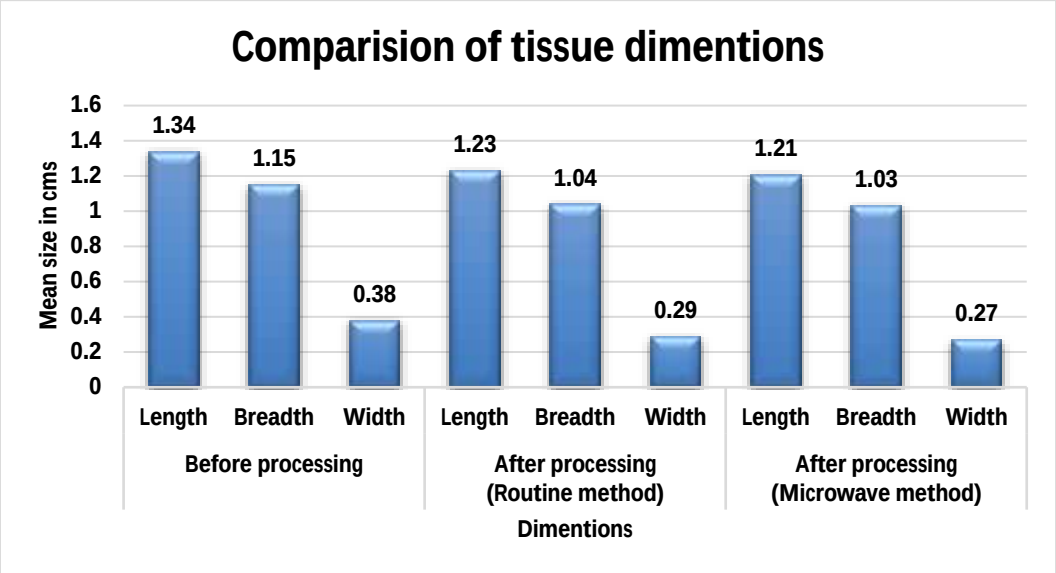

Figure 5.3 Comparison of tissue dimensions

Tissue sections was taken from specimen and cut into two equal halves. The volume of each section was assessed before and after processing for length, breadth and width. (Figure 6.12). Percentage of shrinkage was calculated. The average volume of tissue was 1.34x1.15x0.38= 0.585 cumm i.e. 100% volume of tissue. After tissue processing by routine method, the average volume of tissue was 1.23x1.04x0.29=0.37 cumm. Thus, volume after processing was 63.31% by routine method which showed shrinkage of 100%-63.3%=36.7%.

The tissue processed by microwave method showed mean volume of 1.21x1.03x0.27=0.34 cumm. Thus, volume after processing was 57.5% by microwave method which showed shrinkage of 100%-57.5%=42.5%. Shrinkage of tissue by routine method was 36.7% and by microwave method was 42.5%. Thus, difference of shrinkage between two methods was 42.5%-36.7%=5.8% which showed shrinkage of tissue was slightly more in microwave method than routine tissue processing method.

Microscopic findings:

The evaluation of microscopic slides processed and stained by both methods were based on four parameters and ten subparameters by expert and senior pathologist(Figure 6.13). All tissues were assessed as Grade IV (Excellent)-Marks between 31 to 40.

A. Cellular details:

1. Cellular outline

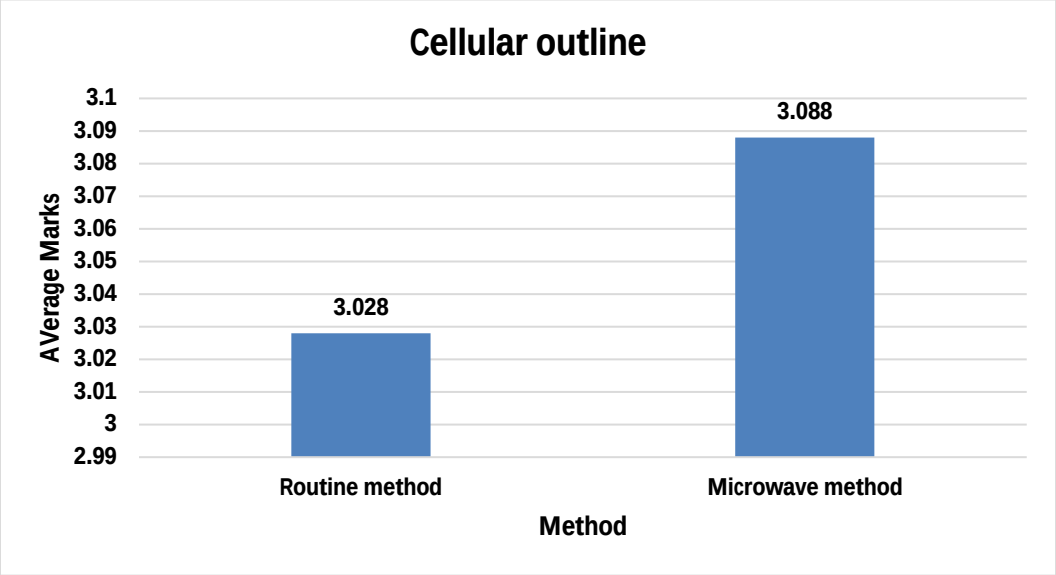

Figure 5.4 comparison of cytoplasmic outline average marks for routine method and microwave method.

Mean score for cellular outline by routine and microwave processed tissues was 3.028±0.17 and 3.088±0.28 respectively. There was a significant difference between the means as an independent sample t-test showed, t (350) = 3.427, (p= 0.0012, $p < 0.05$). This shows statistically significant difference in quality of the slides produced by the two processing methods in cellular detail.

Therefore, according to observations, cellular outline is better seen in domestic microwave processed and stained tissue than routine method tissue.

2. Clarity

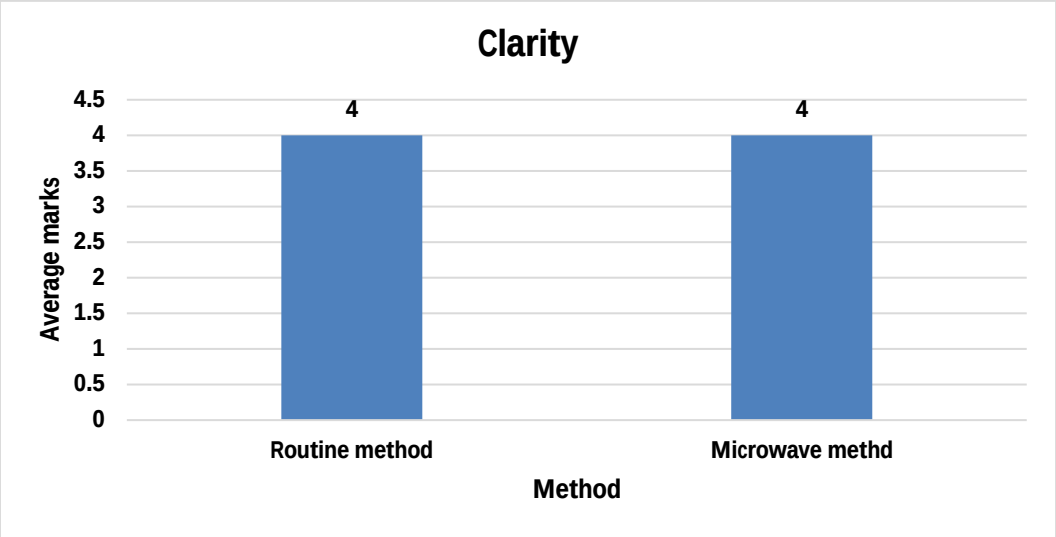

Figure 5.5 Comparison of clarity average marks for routine method and microwave method.

Mean score for clarity by both routine and microwave processed tissues was 4. This shows that clarity of tissue obtained by both the methods are optimal and equal.

3. Integrity of tissue

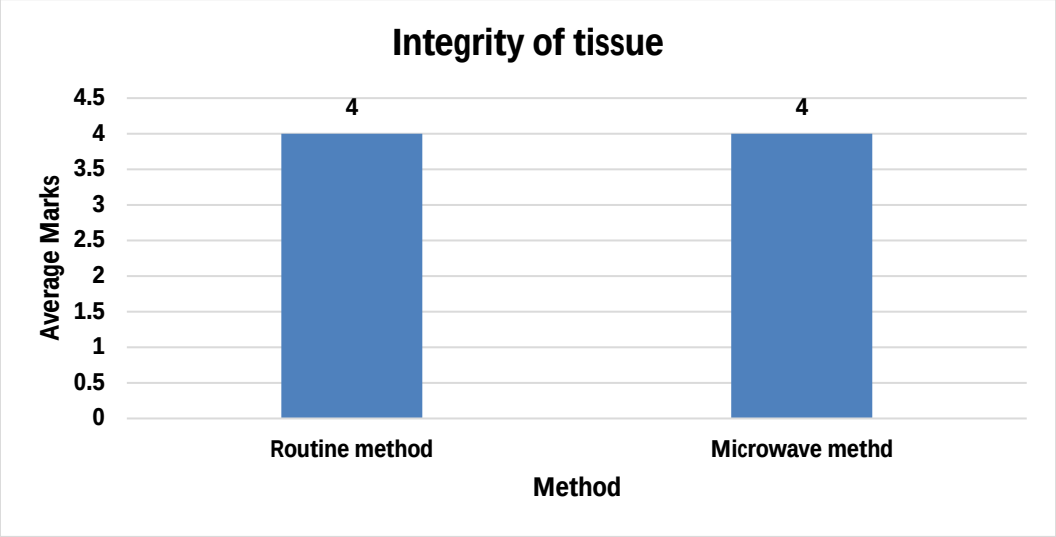

Figure 5.6 Comparison of integrity of tissue average marks for routine method and microwave method.

Mean score for integrity of tissue by routine and microwave processed tissues was 4. This shows that integrity of tissue obtained after both the methods are optimal and equal.

B. Cytoplasmic details

4. Nuclear Cytoplasmic Contrast

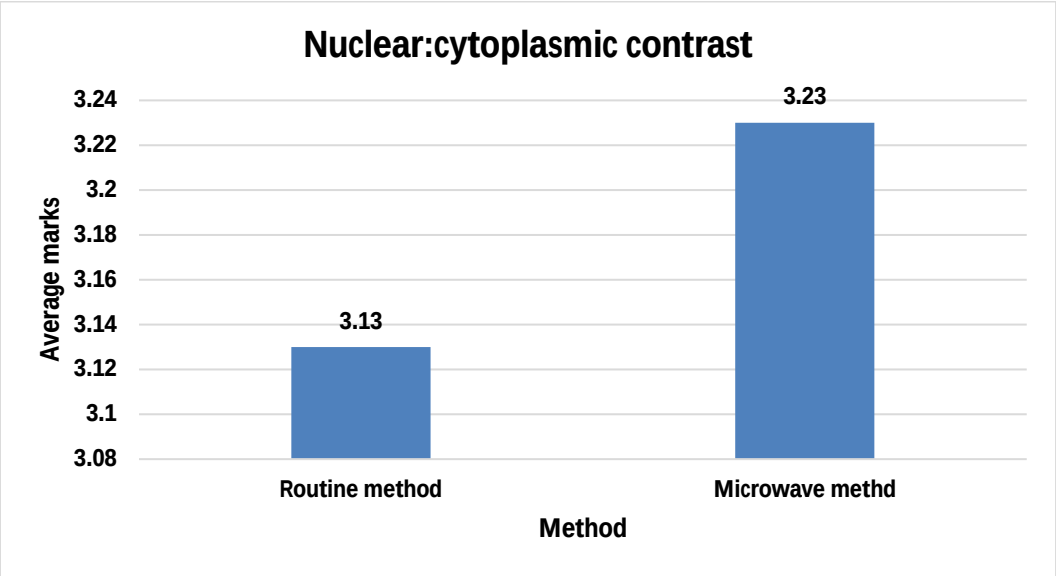

Figure 5.7 Comparison of Nuclear:cytoplasmic contrast average marks for routine method and microwave method.

Mean score for Nuclear:cytoplasmic contrast by routine and microwave processed tissues was 3.13±0.33 and 3.23±0.42 respectively.

There was a significant difference between the means as an independent sample t-test showed, $t(350) = 3.50$, ($p = 0.0009$, $p < 0.05$). This shows statistically significant difference in Nuclear:cytoplasmic contrast produced by the two processing methods.

Therefore, according to observations, Nuclear:cytoplasmic contrast is better seen in domestic microwave processed and stained tissue than routine method tissue.

5. EOSINOPHILIA/ GRANULARITY

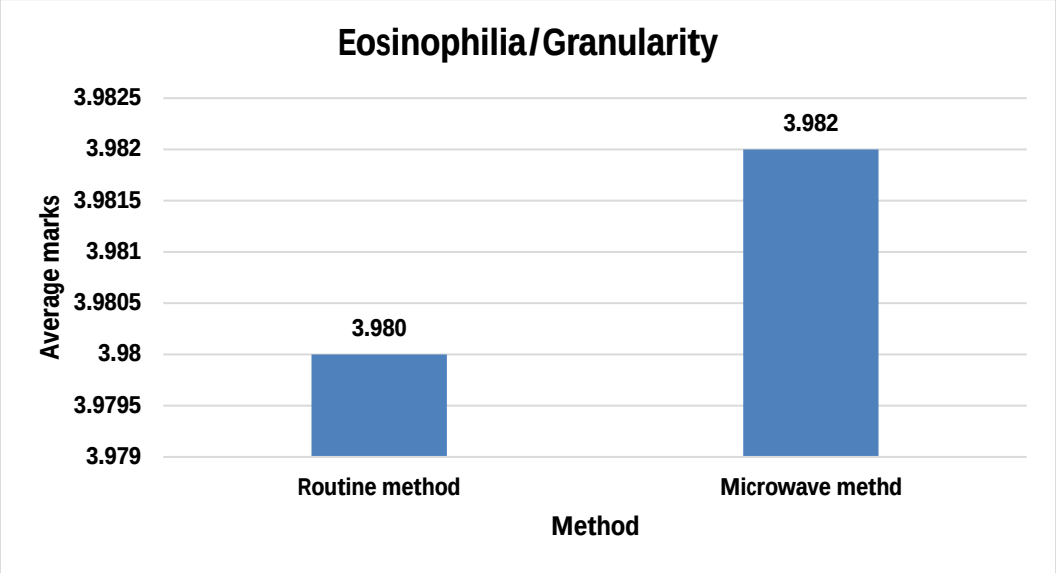

Figure 5.8 Comparison of Eosinophilia/granularity average marks for routine method and microwave method.

Mean score for Eosinophilia/granularity by routine and microwave processed tissues was 3.980±0.14 and 3.982±0.11 respectively.

The independent sample t-test showed, t (350) = 0.21, (p= 0.8335). This shows that there is no statistically significant difference in Eosinophilia/Granularity produced by the two processing methods.

C. NUCLEAR DETAILS

6. Clarity of nucleus & nucleoli

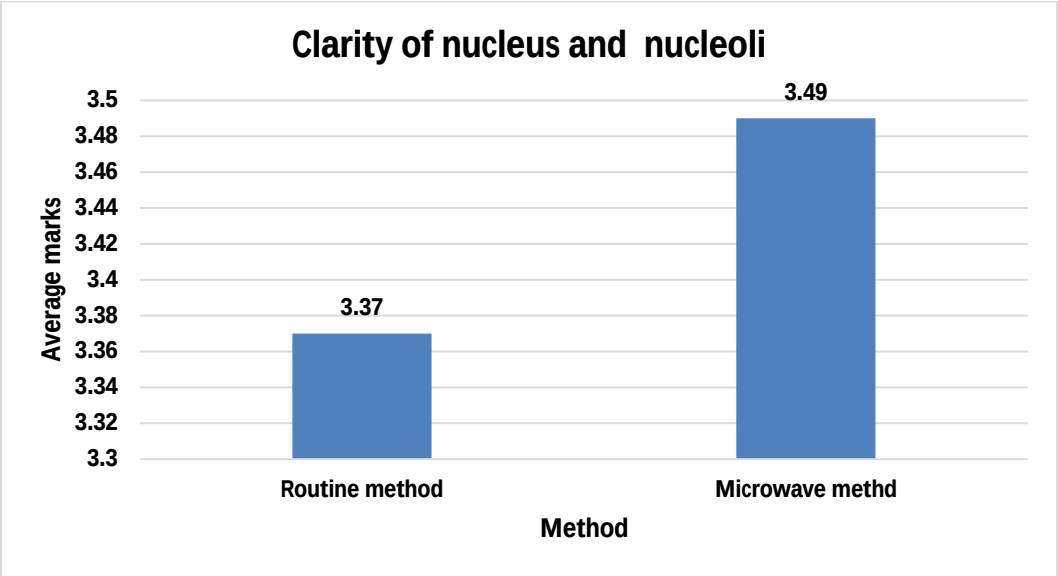

Figure 5.9 Comparison of Clarity of nucleus and nucleoli average marks for routine method and microwave method.

Mean score for Clarity of nucleus and nucleoli by routine and microwave processed tissues was 3.37±0.45 and 3.49±0.50 respectively.

There was a significant difference between the means as an independent sample t-test showed, $t(350) = 3.61$, ($p = 0.0006$, $p < 0.05$). This shows statistically significant difference in clarity of nucleus and nucleoli produced by the two processing methods.

Therefore, according to observations, clarity of nucleus and nucleoli is better seen in domestic microwave processed and stained tissue than routine method tissue.

7. Clarity of nuclear membrane

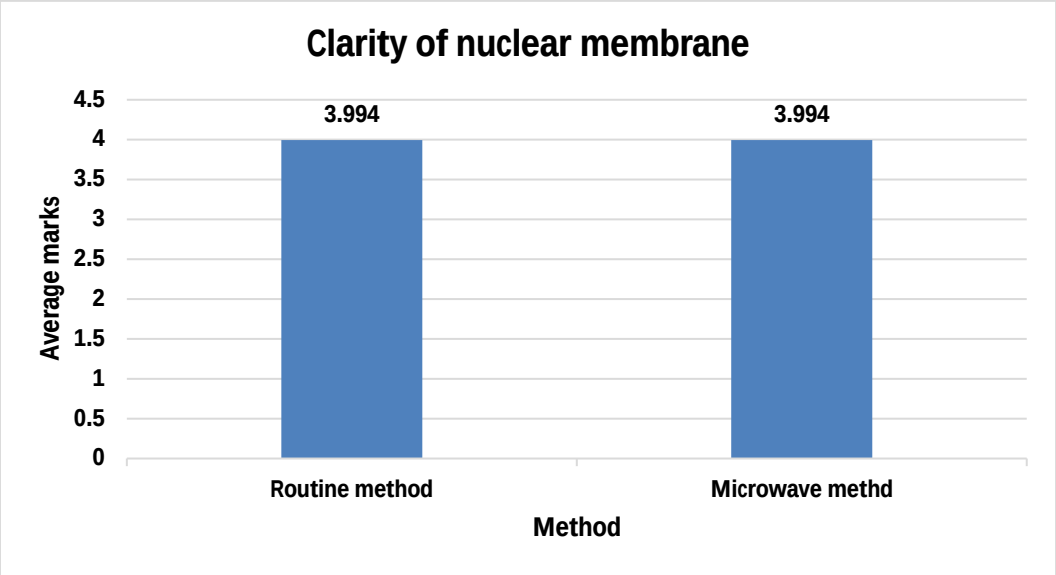

Figure 5.10 Comparison of clarity of nuclear membrane average marks for routine method and microwave method.

Mean score for clarity of nuclear membrane by routine and microwave processed tissues was 3.994. This shows that nuclear membrane is sharp, well preserved and stained equally in both the methods.

8. Clarity of chromatin

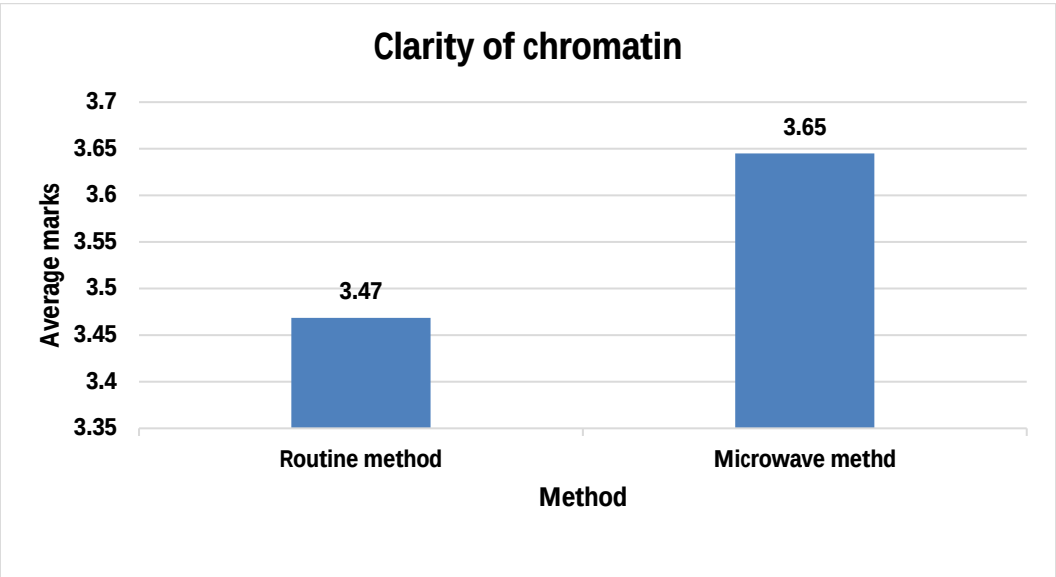

Figure 5.11 comparison of Clarity of chromatin average marks for routine method and microwave method.

Mean score for clarity of chromatin by routine and microwave processed tissues was 3.47±0.50 and 3.65±0.48 respectively. There was a significant difference between the means as an independent sample t-test showed, t (350) = 4.86, (p=0.0001,p<0.05)). This shows statistically significant difference in clarity of chromatin produced by the two processing methods.

Therefore, according to observations, clarity of chromatin is better seen in domestic microwave processed and stained tissue than routine method tissue.

D. Staining characteristics

9. Colour intensity

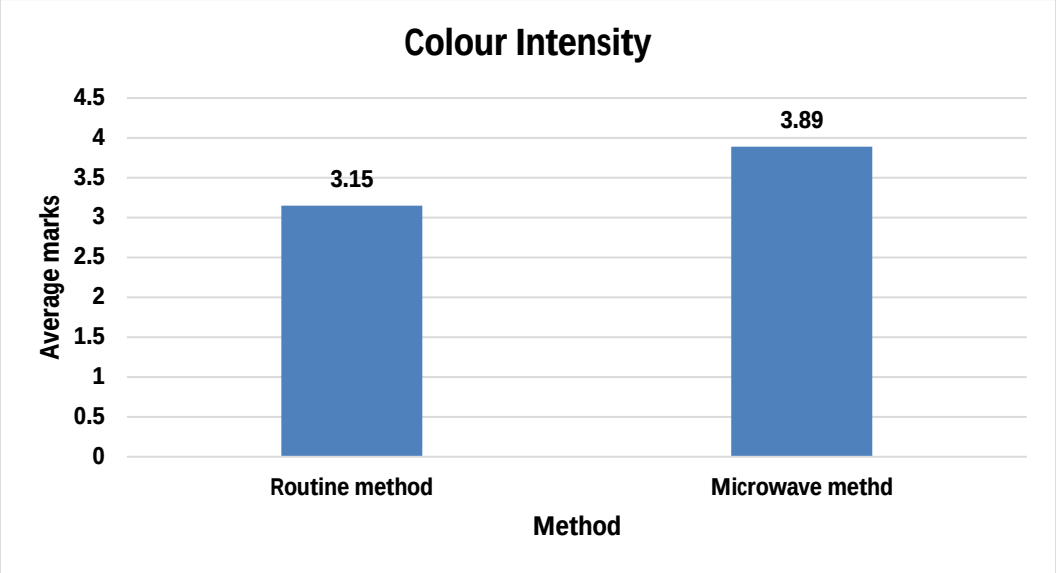

Figure 5.12 comparison of colour intensity average marks for routine method and microwave method.

Mean score for colour intensity by routine and microwave processed tissues was 3.15±0.35 and 3.89±0.32 respectively.

There was a significant difference between the means as an independent sample t-test showed, t (350) = 29.19, (p= 0.0001, p < 0.05). This shows statististically significant difference in colour intensity produced by the two processing methods.

Therefore, according to observations, colour intensity is better seen in domestic microwave processed and stained tissue than routine method tissue.

10. Uniformity

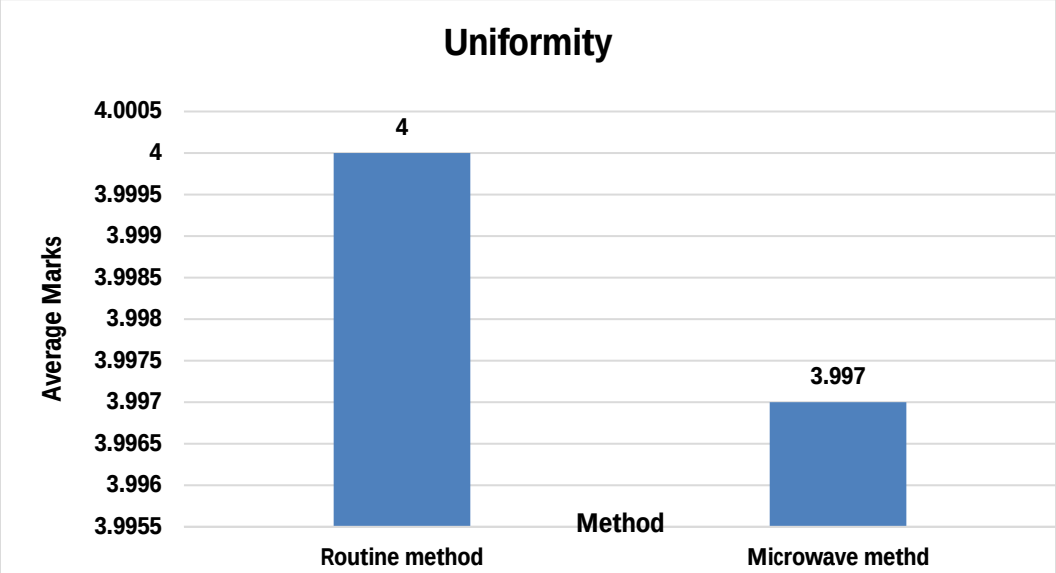

Figure 5.13 Comparison of uniformity average marks for routine method and microwave method.

Mean scores for uniformity by routine and microwave processed tissues was 4 and 3.997±0.053 respectively.

There was difference between the means as an independent sample t-test showed, t (350) = 1.122, (p= 0.5233). This shows that results obtained for uniformity are slightly better by routine method than microwave processed and stained tissue but the difference is not statistically significant as p value is more than 0.05.

SUMMARY TABLE

Table 5.2. Statistical analysis of samples processed by DMWTP and CTP with independent sample t-test

Parameter	Processing method	Mean	Standard Deviation	Standard error of difference	T value	p value	Confidence Interval at 95% level	
Cellular details							**Lower limit**	**Upper limit**
Cellular outline	Routine	3.02	**0.17**	**0.0175**	**3.427**	**0.0012***	**-0.094**	**-0.026**
	Microwave	3.08	**0.28**					
Clarity	Routine	4	**-**	**-**	**-**	**-**	**-**	**-**
	Microwave	4	**-**		**-**	**-**	**-**	**-**
Integrity of tissue	Routine	4	-	-	-	-	-	-
	Microwave	4	-	-	-	-	-	-
Cytoplasmic details								
Nuclear cytoplasmic - contrast	Routine	3.13	**0.33**	**0.0286**	**3.50**	**0.0009***	**-0.156**	**-0.044**
	Microwave	3.23	**0.42**					
Eosinophilia/ Granularity	Routine	3.980	**0.14**	**0.0095**	**0.21**	**0.8335**	**-0.021**	**0.017**
	Microwave	3.982	**0.11**					
Nuclear details								
Clarity of nucleus & nucleoli	Routine	3.37	**0.45**	**0.036**	**3.61**	**0.0006***	**-0.201**	**-0.059**
	Microwave	3.50	**0.50**					
Clarity of nuclear membrane	Routine	3.99	**0.08**	**-**	**-**	**-**	**-0.012**	**0.012**
	Microwave	3.99	**0.08**					
Clarity of chromatin	Routine	3.47	**0.50**	**0.037**	**4.86**	**0.0001***	**-0.257**	**-0.107**
	Microwave	3.65	**0.48**					
Staining characteristics								
Colour intensity	Routine	3.15	**0.35**	**0.0253**	**29.19**	**0.0001***	**-0.789**	**-0.690**
	Microwave	3.89	**0.32**					
Uniformity	Routine	4	**-**	**0.0027**	**1.122**	**0.5233**	**-**	**-**
	Microwave	3.997	**0.05**					

· *Statistically significant

Thus, analysis of data was done which showed following results.

- Cellular outline, nuclear cytoplasmic contrast, clarity of nucleus and nucleoli, clarity of chromatin and colour intensity were better in microwave method as compared to routine method and the difference was statistically significant.

- Clarity of cellular details, integrity of tissue and clarity of nuclear membrane were equally preserved in both the methods.

- There was slight difference in Eosinophilia/granularity and uniformity in staining by both the methods. However the difference was not statistically significant.

Special stain:

- In Histopathology, the major part of the routine work consists of examination of slides stained by hematoxylin and eosin stain. But some of the tissue components can not be discriminated by routine hematoxylin and eosin stain.

- Various special stains like Periodic acid Schiff, Alcian blue, Massion trichrome, Fontana Massion were performed on tissues processed by both the methods and the results were comparable (Figure 6.14). Thus, tissue components retain their specific chemical characteristics not only by routine processing but also by microwave processing.

Immunohistochemistry:

- Immunohistochemistry procedure is a technique for recognizing cellular or tissue constituents (antigens) by means of antigen-antibody reaction.

- The immunohistochemistry was performed on tissues processed by both the methods and the results were comparable (Figure 6.15). Thus, antigens of tissues were preserved in microwave processing.

Table 5.3 Comparison of time taken in processing of tissues by routine and microwave method

Stages	Routine method	Microwave method
Running water		5 minutes
Dehydration	10 hours	42 minutes
Clearing	3 hours	-
Impregnation	3 hours	20 minutes
Total time required	**16 hours**	**67 minutes**

Table 5.4 Comparison of time taken in Staining of tissues by routine and microwave method

Stages	Routine method (Minutes)	Microwave method (Minutes)
Running tap water	2 minutes	2 minutes
Hematoxylin stain	3 minutes	30 seconds
Running tap water	3 minutes	30 seconds
Differentiation by 1% acid alcohol	Dip for 2-3 seconds	1 drop for 2-3 seconds
Running tap water	5 minutes	30 seconds
Tissue dipped in petridish containing tap water in microwave at 180 W	-	30 seconds
Eosin stain	Dip	1 drop for 2-3 seconds
Isopropyl alcohol	Dip	Few drops for 2-3 seconds
Xylene	10 minutes	10 minutes
Total staining time	**23 minutes**	**14 minutes**

Table 5.5 Overall working time for the two methods.

	Routine method	Microwave method
Total time	**16 hours 23 minutes**	**81 minutes**

The time taken during the processing and staining of tissues by both methods were recorded and tabulated. The routine tissue processing was performed by dip and dunk tissue processor and staining by manual method. The overall processing time by Routine method is 16 hours whereas the overall processing time by domestic microwave was only 67 minutes. Time taken during staining by manual method takes 23 minutes while domestic microwave method takes 14 minutes for staining.

The reagents utilised in routine method consists of 11 buckets of 2000 ml (2 litre) capacity which can process approximately 20 tissues at a time optimally. While microwave method utilises 6 buckets reagents of 300 ml each that can process 4 tissues at a time optimally. As routine method is performed on room temperature, evaporation of reagents are minimal. In contrast, in each 10 minutes cycle of microwave oven approximately 30-50 ml reagents are evaporated. Thus, Evaporation of reagent is comparatively more in microwave method.

Overall processing and staining time by routine method was 16 hours and 23 minutes while time taken by microwave assisted tissue processing and staining method was 81minutes.Though it has its limitations, the domestic microwave method has a significantly shorter processing and staining time.