CHAPTER 4

RESULTS

4.1 Phytochemical Detection by (TLC) on *Rhodomyrtus tomentosa* extracts

Crude plant extracts are always composed of numerous compounds known as phytochemicals that are responsible for biosynthesis, metabolism and biological function in plant. In this study, analysis of phytochemical compounds in four different *R. tomentosa* extracts was carried out by means of thin layer chromatography (TLC). TLC is a useful method in fractionation, separation and detection of compounds. This method is used very widely for qualitative analysis of plant extracts. Special spraying reagents such as vanillin and dragendorff were used because of its ability to detect wide range of compounds. At the beginning of analysis, several mobile systems were tested to get the best separation for each extracts before the actual analysis can be done. A combination of different polarity solvents (toulene-ether, 70:30; toluene-ethylacetate, 60:40, chloroform-methanol, 50:50) were used as solvent system were found to be appropriate in separating these compounds from the extract. Alkaloids, phenols and terpenoids were indentified in all extracts solvent whereas chlorophylls were present only in methanol, chloroform and petroleum ether extracts.

Table 4.1.1: TLC analysis of crude water extract from the fruits of *Rhodomyrtus tomentosa*

Labelled Compound	R_f in solvent			Visible light	Spraying reagents			Comment
	Toulene-ether (70:30)	Toluene-EtoAc (60:40)	Chloroform-methanol (50:50)		Dragendorffs	10% Vanillin in H_2SO_4	Aniseldehyde in H_2SO_4	
W11	-	0.377	-	-	-	Purple (+)	-	Terpenoid
W10	0.406	-	0.374	-	-	Purple (++++)	-	Terpenoid
W9	0.382	0.351	-	Orange (+)			Blue (++)	Phenol
W8	0.355	-	0.340	-			Blue (+)	Phenol
W7	-	0.223	-	Yellowish green (+)	-	-	Brown (++)	Phenol
W6	0.197	-			-	-	Blue (+)	Phenol
W5	-	0.183	-	-	-	Purple (++++)	-	Terpenoid
W4	0.166	0.172	-	Orange (+)	Yellow (++)	-	-	Alkaloid
W3	-	-	0.338		-	-	Blue (+++)	Phenol
W2	-	0.152	0.331	-	-	-	Brown (+)	Phenol
W1	0.100	0.122	0.125	Red (++)	-	-	Brown (++)	Phenol

Indication of colour intensity: Lightest (+) Light (++) Dark (+++) Darkest (++++)

Table 4.1.2: TLC analysis of crude methanol extract from the fruits of *Rhodomyrtus tomentosa*

Labelled Compound	R_f in solvent			Visible light	Spraying reagents			Comment
	Toulene-ether (70:30)	Toluene-EtoAc (60:40)	Chloroform-methanol (50:50)		Dragendorffs	10% Vanillin in H_2SO_4	Aniseldehyde in H_2SO_4	
M12	0.840	0.782	-	-	-	Bluish black (+++)	-	Terpenoid
M11	0.756	-	0.880	-	Orange (+++)	-	-	Alkaloid
M10	0.628	0.741	-	-	-	Yellow (+++)	-	Terpenoid
M9	-	-	0.519	-	Orange (+++)	-	-	Alkaloid
M8	0.589	0.594	-	Orange (++)	-	-	Purple (++++)	Phenol
M7	-	0.473	0.499	-	-	Purple (++++)	-	Terpenoid
M6	0.333	0.326	0.367		-	Purple (++)	-	Terpenoid
M5	-	-	0.210	Green (++)	-	-	-	Chlorophyll
M4	0.200	0.205	-	-	-	Blue (+++)	-	Terpenoid
M3	-	-	0.178	-	Orange (+++)	-	-	Alkaloid
M2	0.143	-	0.157	Green (++)	-	-	Blue (++)	Phenol
M1	0.125	0.199	0.136	Yellow	-	-	Blue (+)	Phenol

Indication of colour intensity: Lightest (+) Light (++) Dark (+++) Darkest (++++)

Table 4.1.3: TLC analysis of crude chloroform extract from the fruits of *Rhodomyrtus tomentosa*

Labelled Compound	R_f in solvent			Visible light	Spraying reagents			Comment
	Toulene-ether (70:30)	Toluene-EtoAc (60:40)	Chloroform-methanol (50:50)		Dragendorffs	10% Vanillin in H_2SO_4	Aniseldehyde in H_2SO_4	
C13	-	-	0.81	-	-	Purplish blue (+)	-	Terpenoid
C12	-	0.794	0.773	-	-	Purple (+)	-	Terpenoid
C11	0.639	0.788	-	Yellow(++)	Yellow (+)	-	-	Alkaloid
C10	-	-	0.755	-		Brown (++)	-	Terpenoid
C9	-	0.722		Yellow (++)	-	Purple (++++)	-	Terpenoid
C8	0.535	-	0.694	-	Orange (+++)	-	-	Alkaloid
C7	0.511	0.672	0.686	-	-	Bluish black (+++)	-	Terpenoid
C6	-	0.613	0.502	Green (++)	-	-	-	Chlorophyll
C5	-	-	0.446	-	-	-	Blue (++)	Phenol
C4	0.487	0.522	-	-	-	Greenish blue	-	Terpenoid
C3	0.395	0.266	0.350	-	-	-	Blue (+)	Phenol
C2	-	0.197	0.287	Yellow (++)	Orange (+++)	-		Alkaloid
C1	0.243	-	-	Purplish Orange (+)	Yellowish/brown (++)	-	-	Alkaloid

Indication of colour intensity: Lightest (+) Light (++) Dark (+++) Darkest (++++)

Table 4.1.4: TLC analysis of crude petroleum ether extract from the fruits of *Rhodomyrtus tomentosa*

Labelled Compound	R_f in solvent			Visible light	Spraying reagents			Comment
	Toulene-ether (70:30)	Toluene-EtoAc (60:40)	Chloroform-methanol (50:50)		Dragendorffs	10% Vanillin in H_2SO_4	Aniseldehyde in H_2SO_4	
P9	0.912	0.900	-	-	-	Purple (++)	-	Terpenoid
P8	-	0.855	-	Yellow	-	Purple (+)	-	Terpenoid
P7	0.741	-	-	-	-	-	Purple (++)	Phenol
P6	-	0.617	-	-	-	Bluish (+++)	-	Terpenoid
P5	0.570	0.544	0.447	-	-	-	Blue (+)	Phenol
P4	-	0.525	-	Brown	Orange (++++)	-	-	Alkaloid
P3	0.340	-	-	Yellow (++)	-	Purple (++)	-	Terpenooid
P2	0.249	0.218	0.195	Green (++)	-	-	-	Chlorophyll
P1	0.188	0.153	0.160	Yellowish green (+)	-	-	-	Chlorophyll

Indication of colour intensity: Lightest (+) Light (++) Dark (+++) Darkest (++++)

4.11 Histology examination of Hematoxyline and Eosin (H and E)

4.11.1 Histology examination of aorta

Normal group

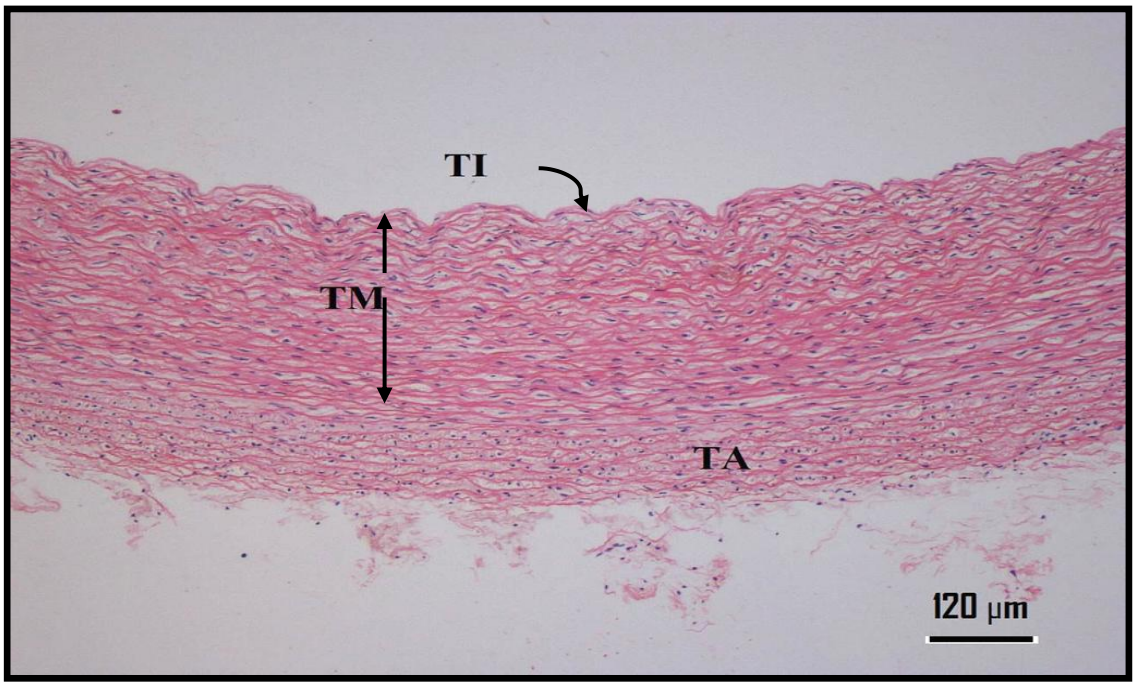


Figure 4.11.1: Cross section of aorta tissue specimen from normal group stained with H&E at LM X 10. TI = tunica intima TM = tunica media TA = tunica adventitia.

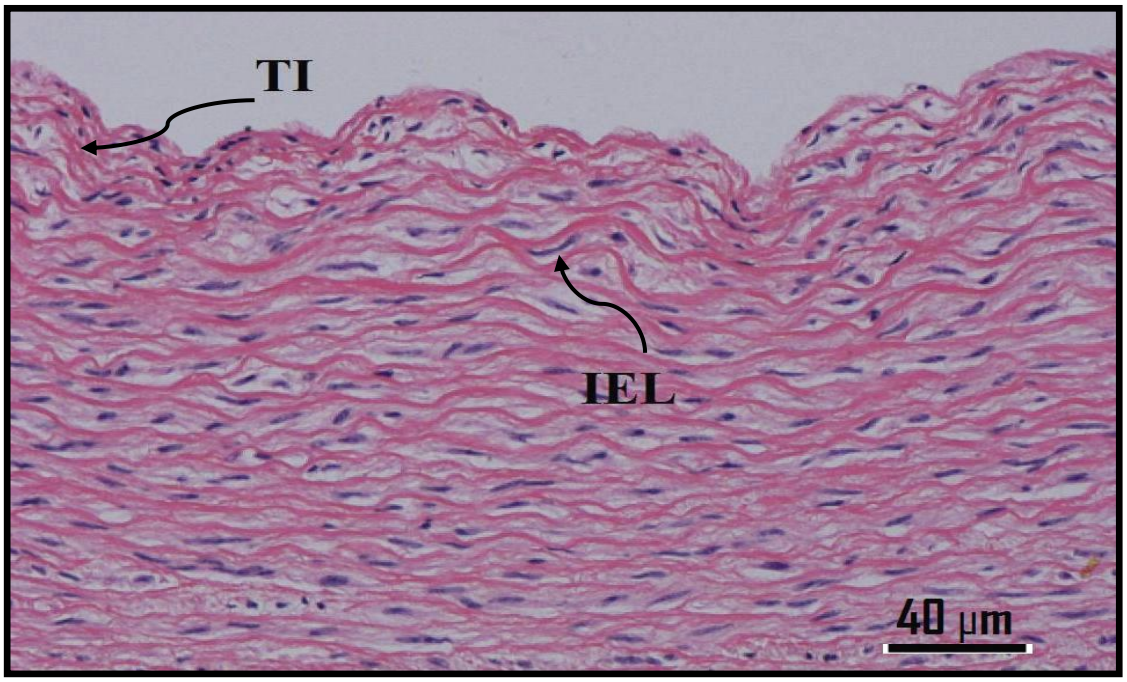


Figure 4.11.2: Cross section of aorta tissue specimen from normal group stained with H&E at LM X 20. TI = tunica intima IEL = internal elastic laminae.

Cholesterol group

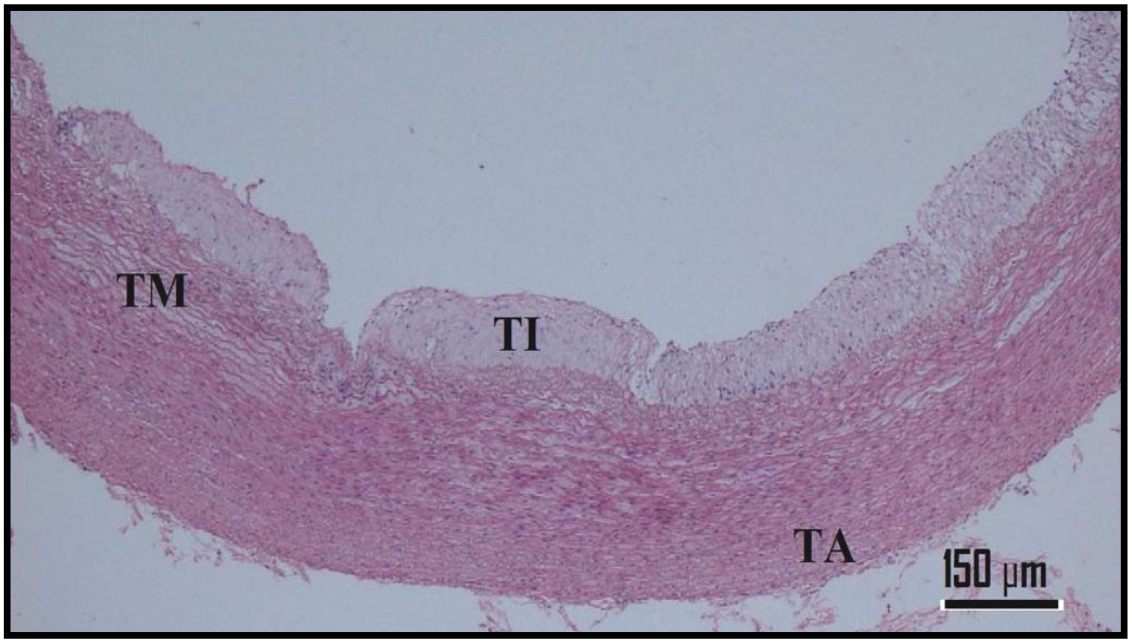


Figure 4.11.3: Cross section of aorta tissue specimen from cholesterol group stained with H&E at LM X 4.TI = tunica intima TM = tunina media TA = tunica adventitia.

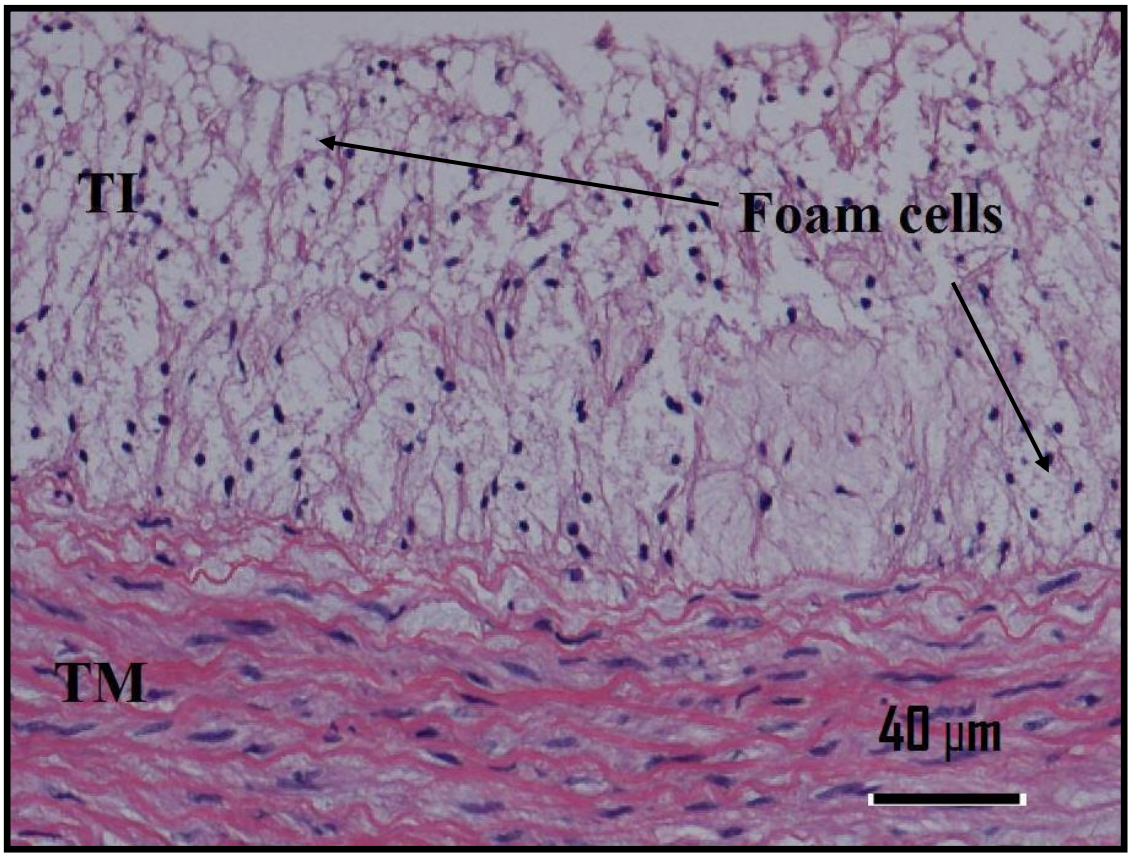


Figure 4.11.4: Cross section of aorta tissue specimen from cholesterol group stained with H&E at LM X 20. TI = tunica intima TM = tunica media.

***R. tomentosa* group**

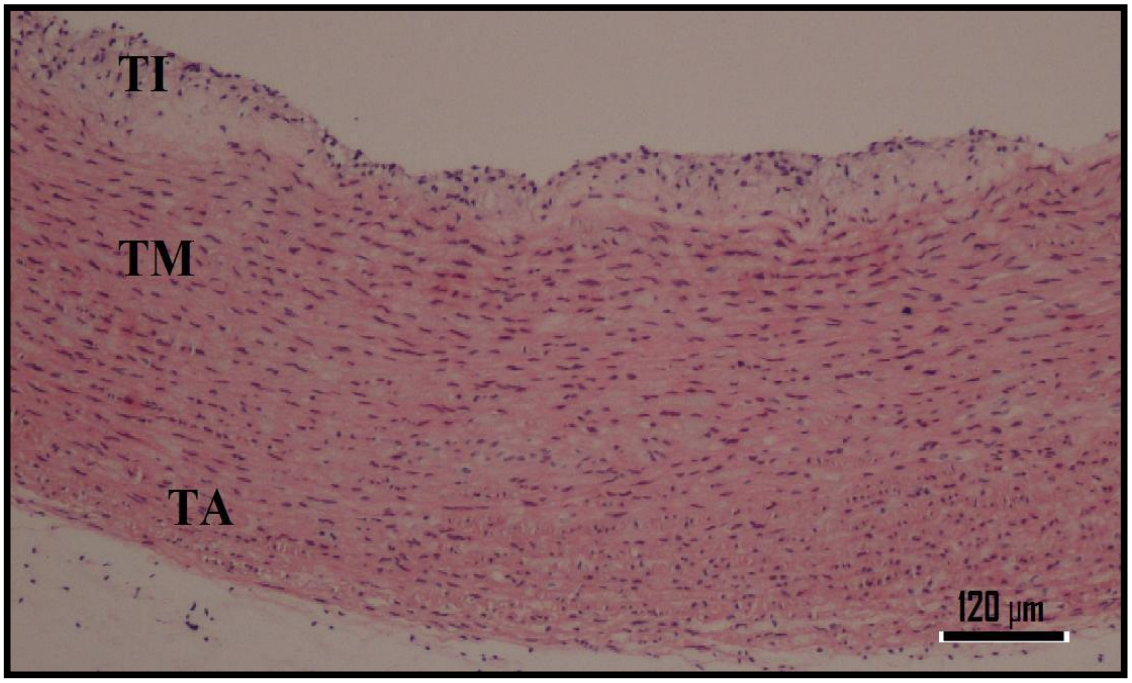


Figure 4.11.5: Cross section of aorta tissue specimen from *R. tomentosa* group stained with H&E at LM X 10. TI = tunica intima TM = tunica media TA = tunica adventitia.

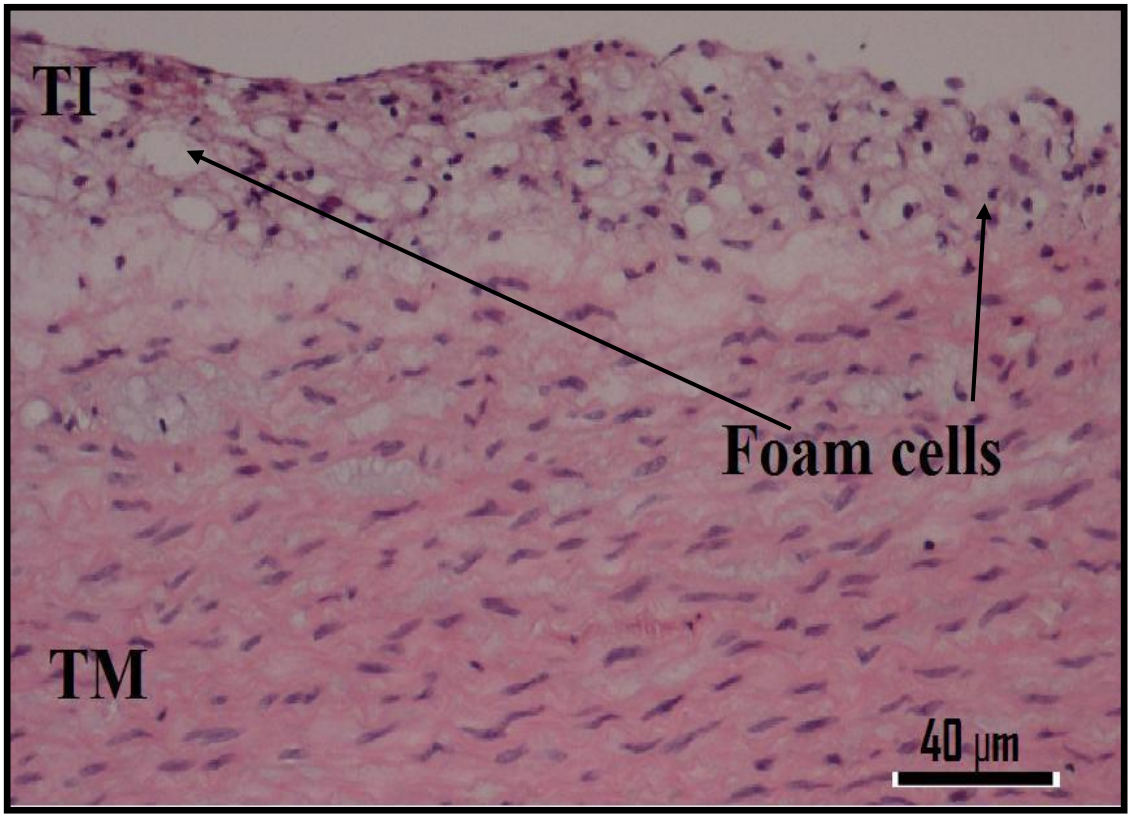


Figure 4.11.6: Cross section of aorta tissue specimen from *R. tomentosa* group stained with H&E at LM X 20. TI = tunica intima TM = tunica media.

Simvastatin group

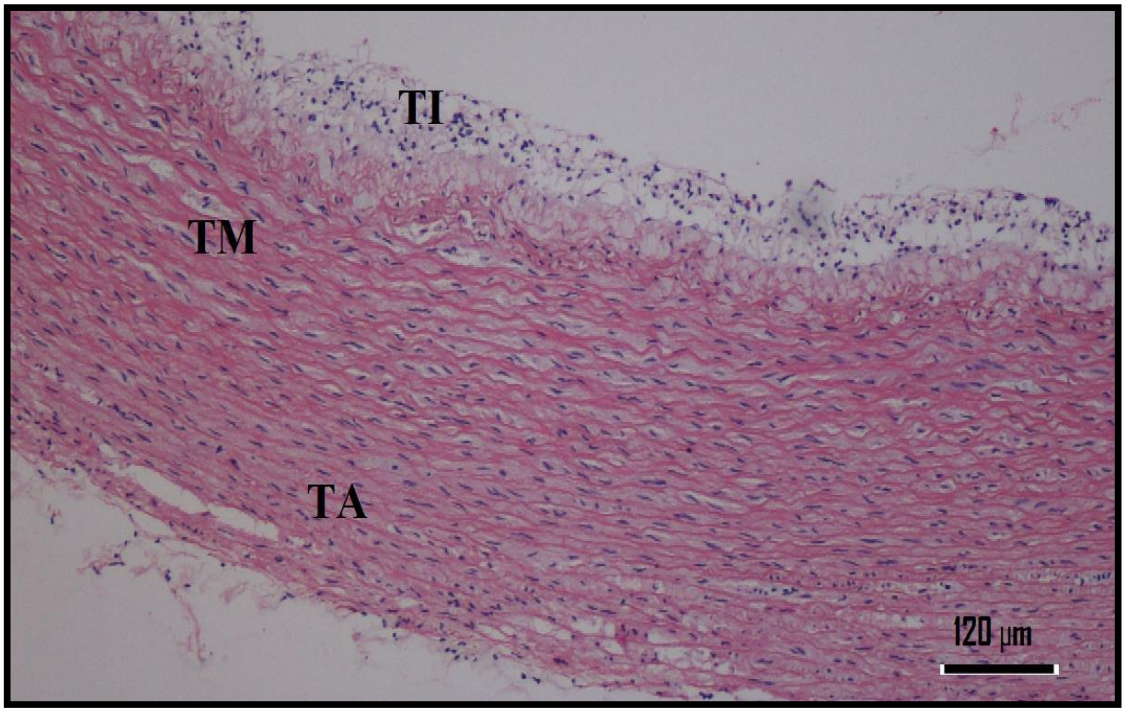


Figure 4.11.7: Cross section of aorta tissue specimen from simvastatin group stained with H&E at LM X 10. TI = tunica intima TM = tunica media TA = tunica adventitia.

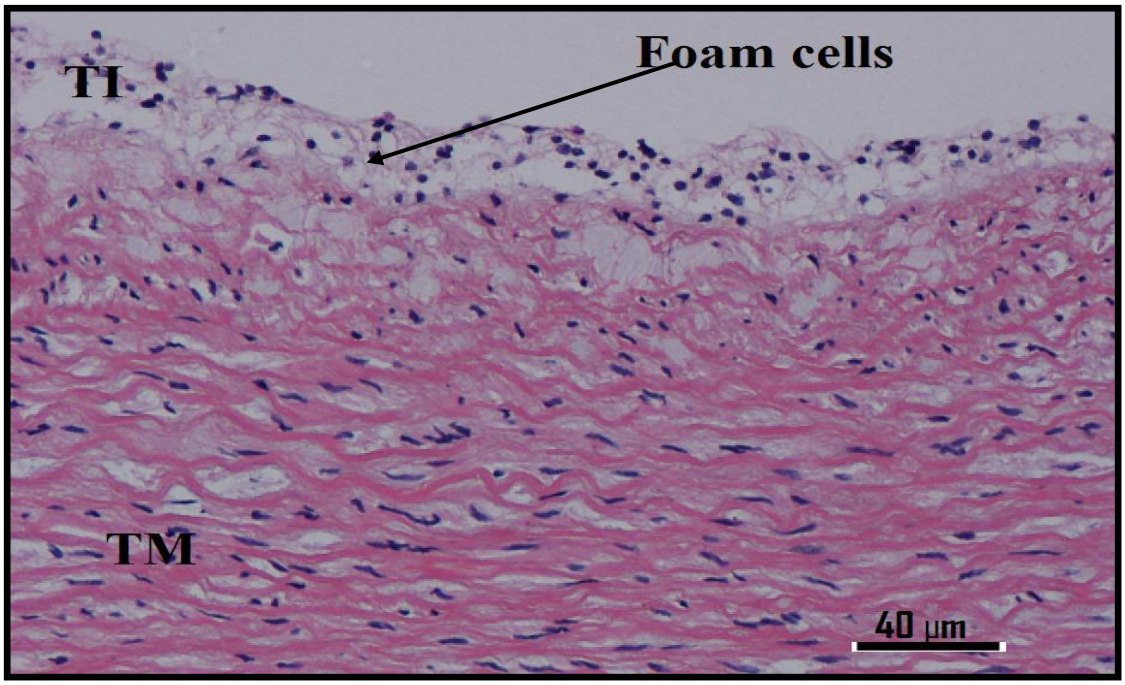


Figure 4.11.8: Cross section of aorta tissue specimen from simvastatin group stained with H&E at LM X 20. TI = tunica intima TM = tunica media TA = tunica adventitia.

4.11.2 Histology examination of Kidney

Normal group

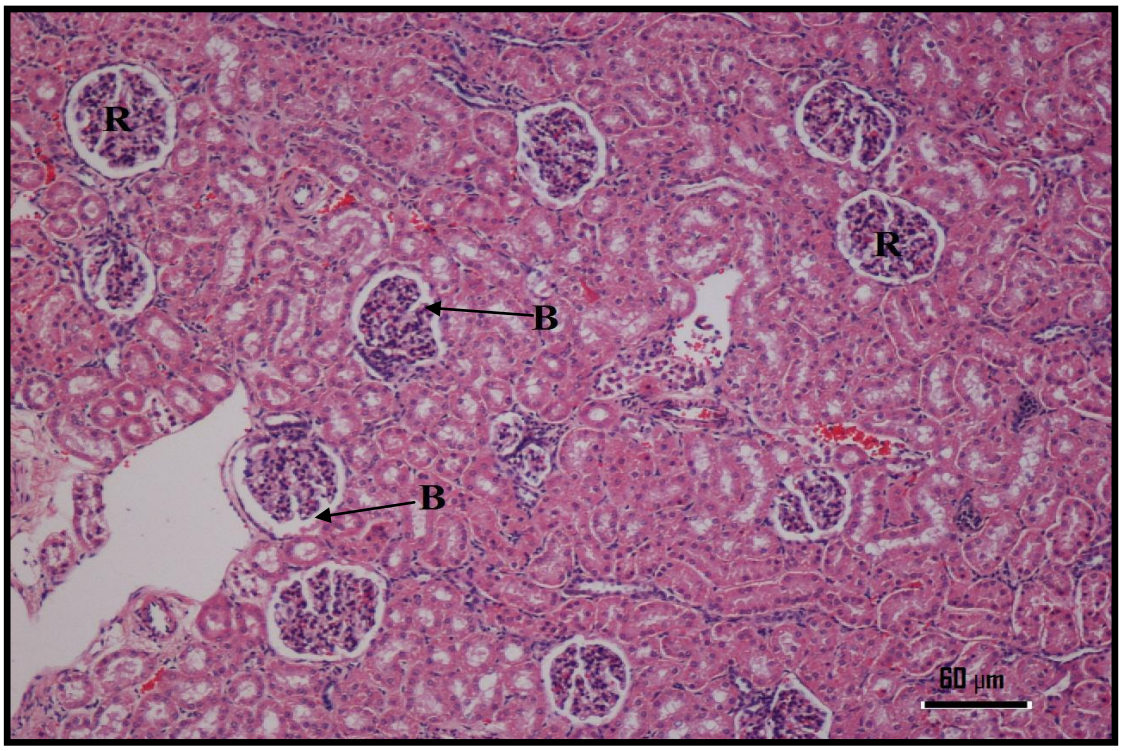


Figure 4.11.9: Cross section of kidney tissue specimen from normal group with H&E at LM X 10. B = Bowman's spaces R= Renal corpuscle

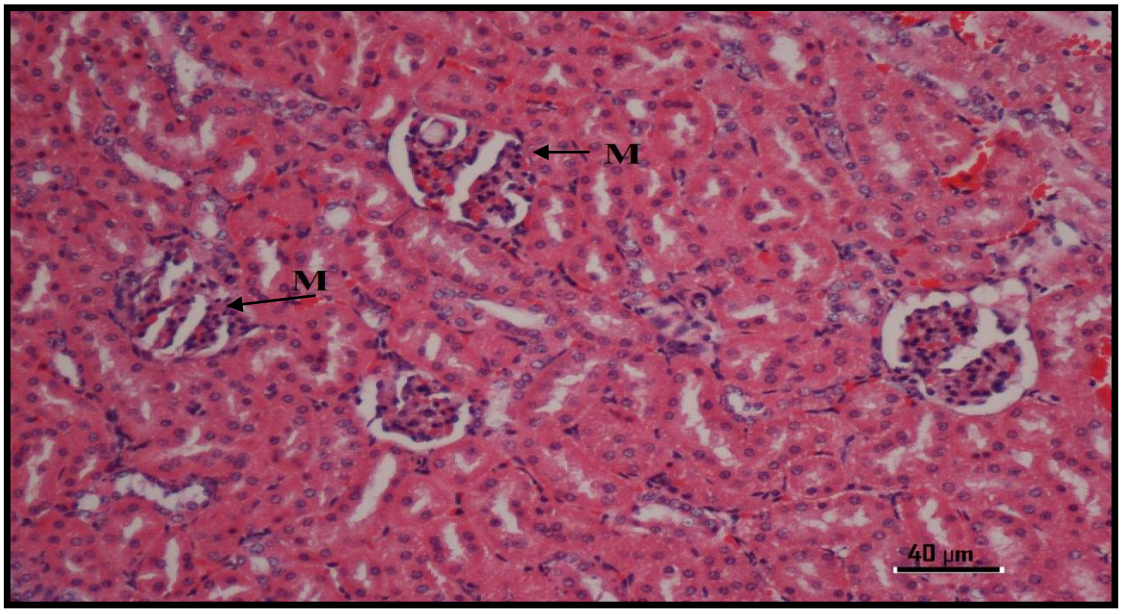


Figure 4.11.10: Cross section of kidney tissue specimen from normal group with H&E at LM X 20. M = mesengial cells

Cholesterol group.

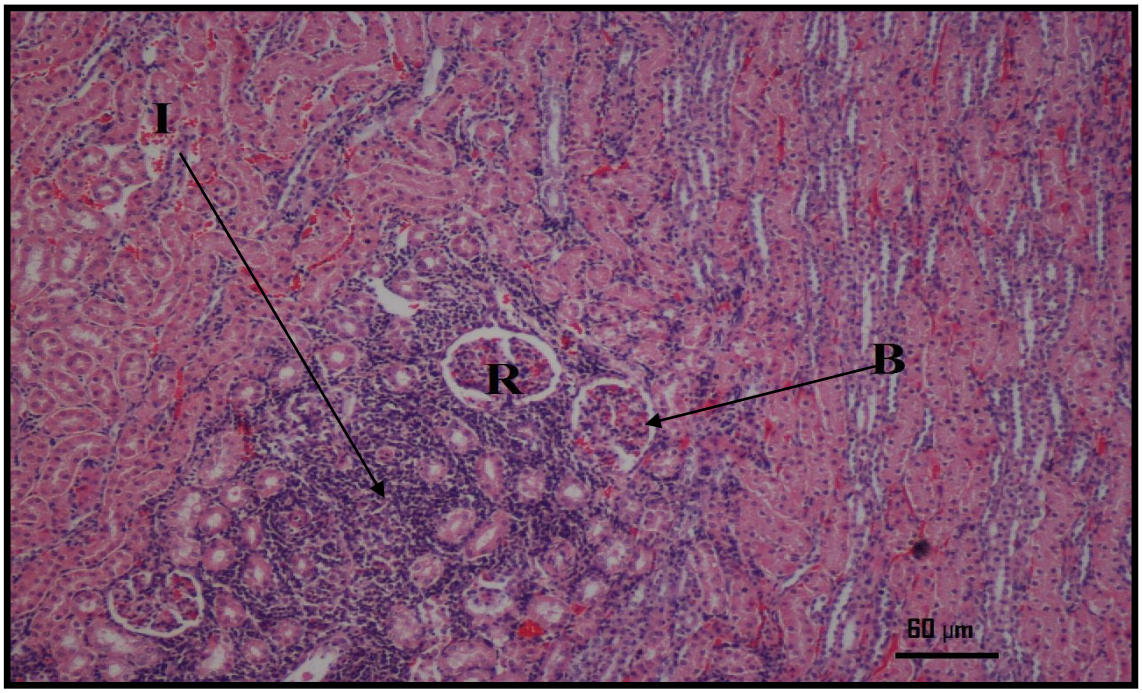


Figure 4.11.11: Cross section of kidney tissue specimen from cholesterol group with H&E at LM X 10. R = renal corpuscle B= Bowman's spaces I= Inflammatory cells

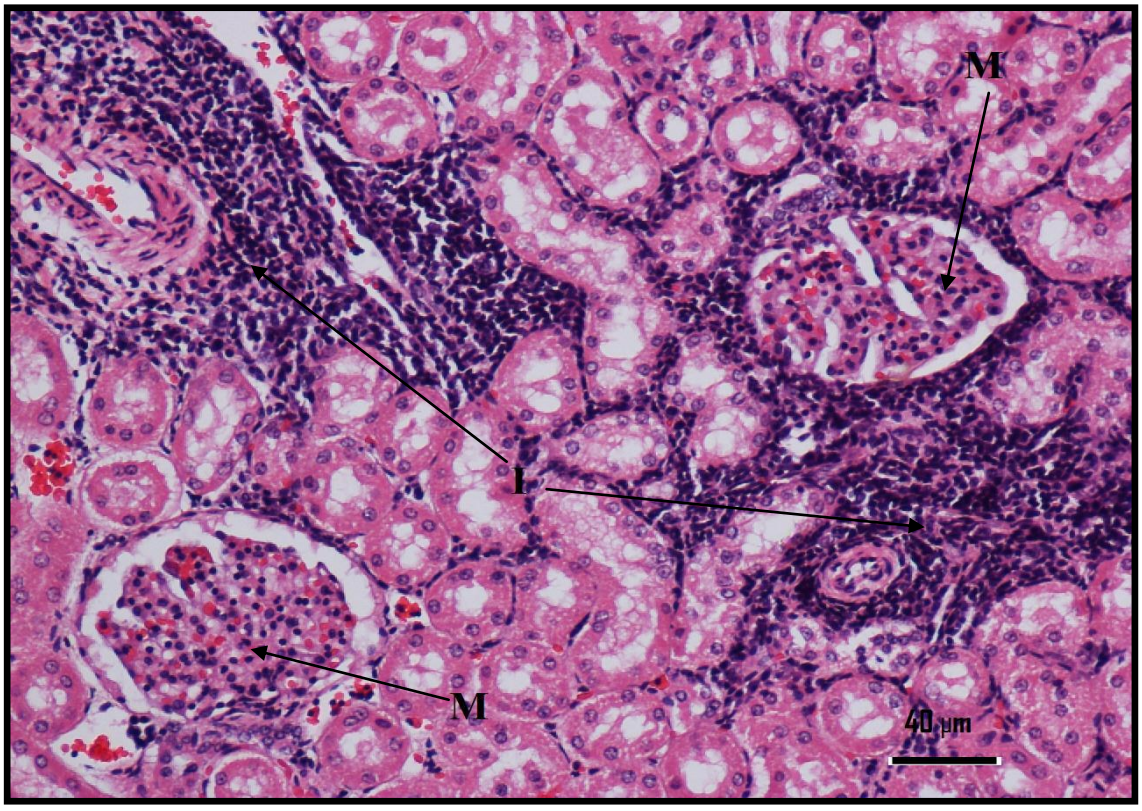


Figure 4.11.12: Cross section of kidney tissue specimen from cholesterol group with H&E at LM X 20. M = mesengial cells I = inflammatory cells

***R. tomentosa* group**

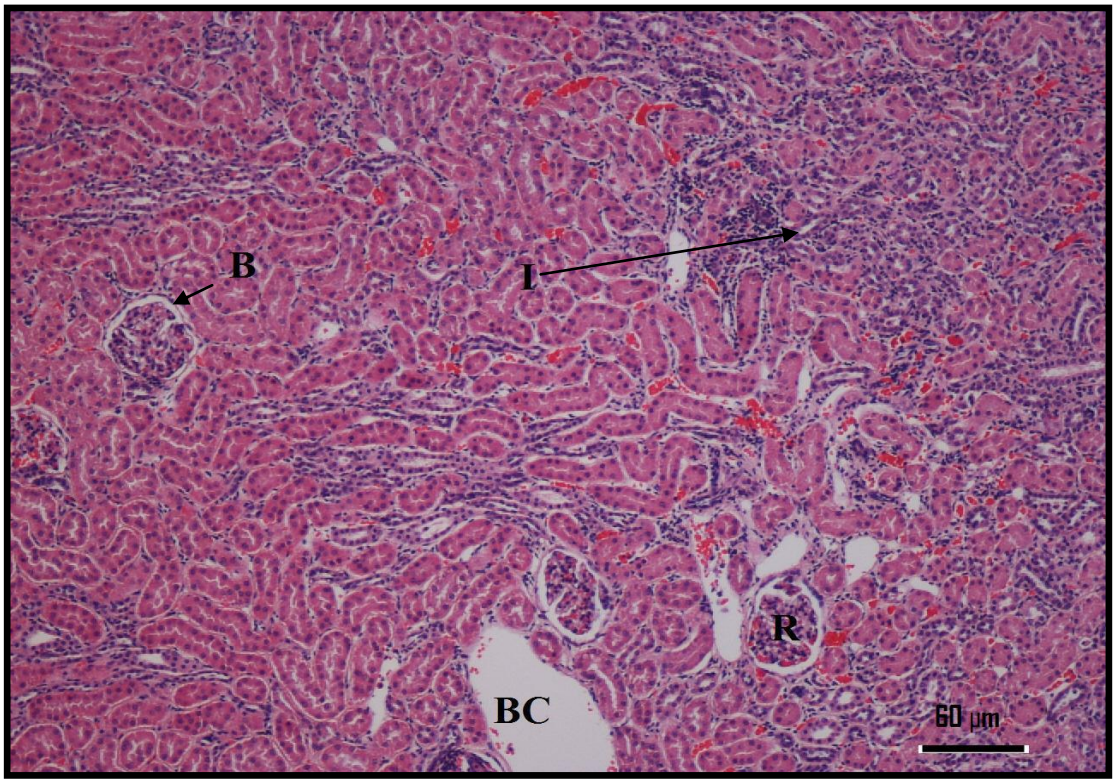


Figure 4.11.13: Cross section of kidney tissue specimen from *R. tomentosa* group with H&E at LM X 10. R= renal corpuscle B= Bowman's spaces I= Inflammatory cells BC = Blood capillary

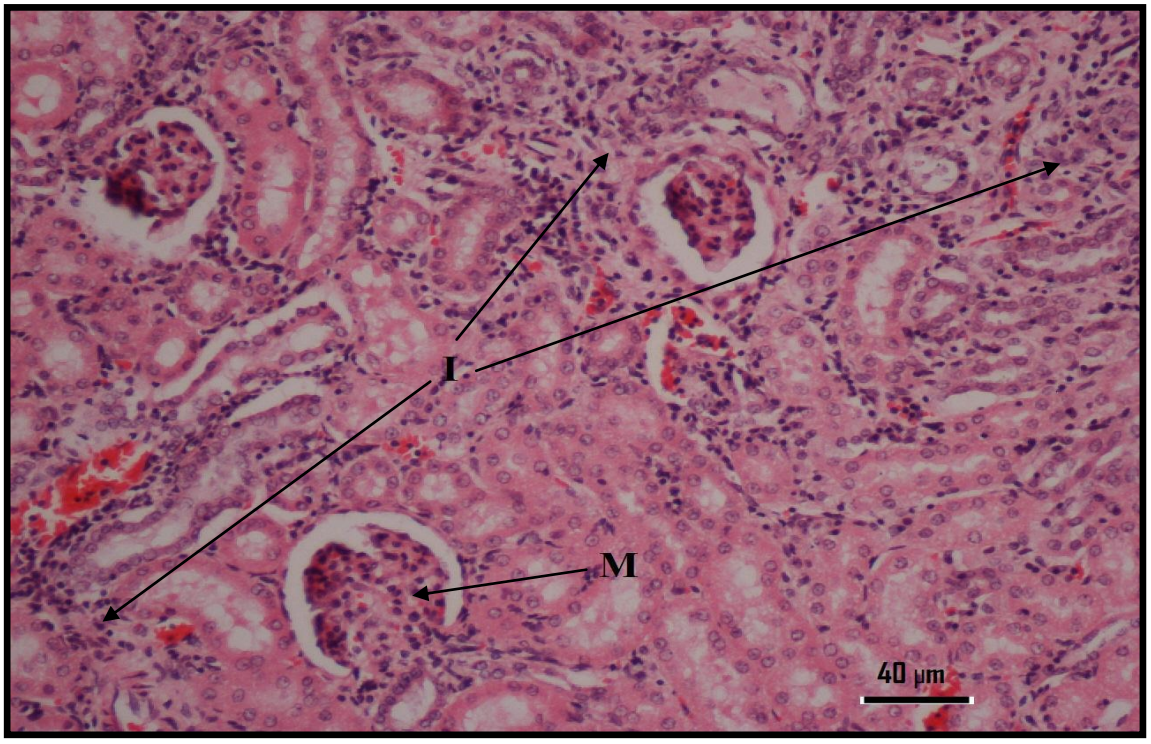


Figure 4.11.14: Cross section of kidney tissue specimen from *R. tomentosa* group with H&E at LM X 20. M = mesengial cells I = inflammatory cells

Simvastatin group

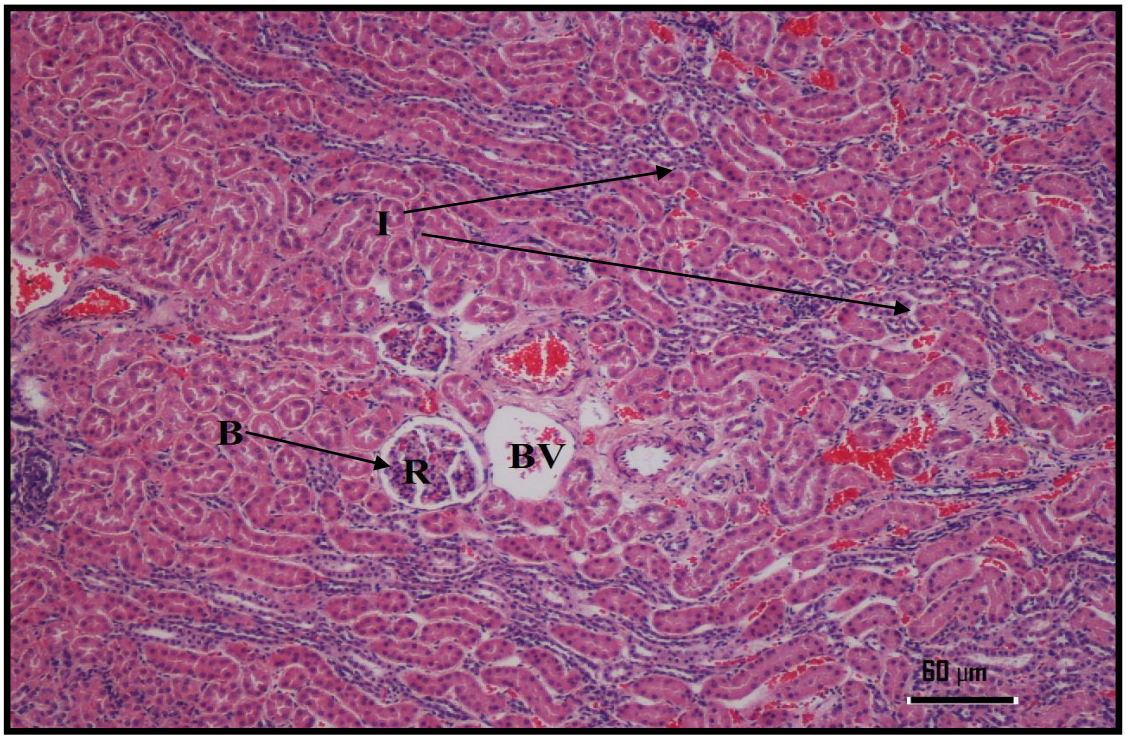


Figure 4.11.15: Cross section of kidney tissue specimen from simvastatin group with H&E at LM X 10. R = renal corpuscle B= Bowman's spaces I= Inflammatory cells BV = Blood vessel

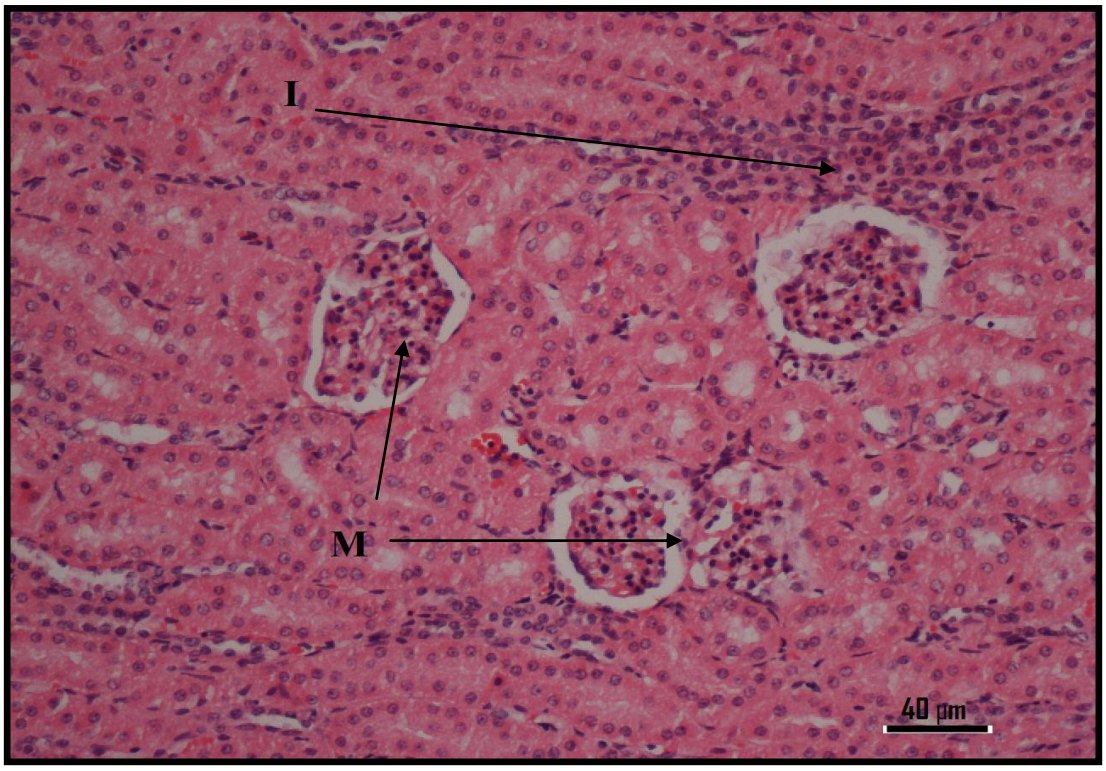


Figure 4.11.16: Cross section of kidney tissue specimen from simvastatin group with H&E at LM X 10. M = mesengial cells I = inflammatory cells

4.11.3 Histology examination of liver.

Normal group

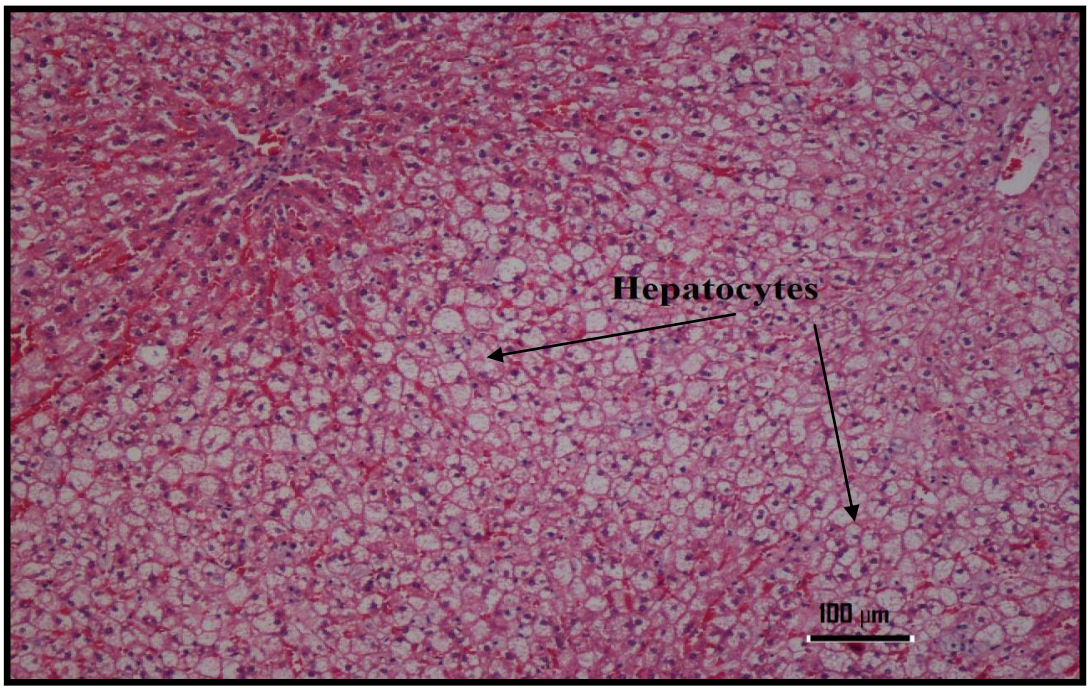


Figure 4.11.17: Cross section of liver tissue specimen from normal group with H&E at LM X 10.

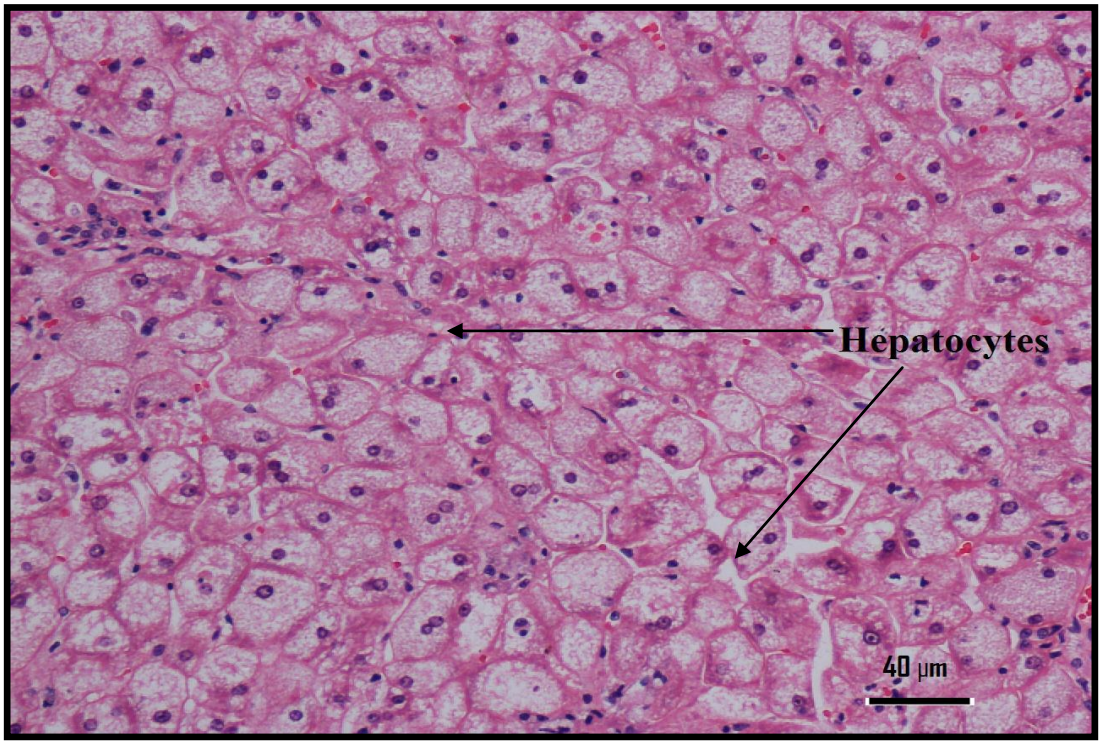


Figure 4.11.18: Cross section of liver tissue specimen from normal group with H&E at LM X 20.

Cholesterol group

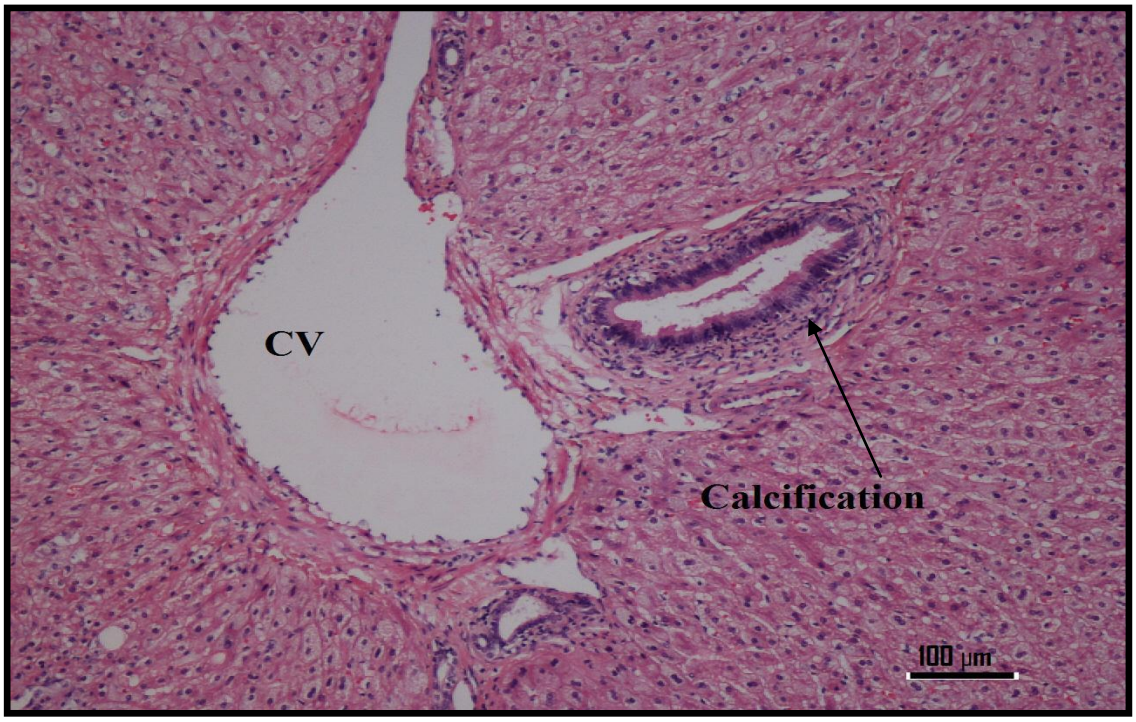


Figure 4.11.19: Cross section of liver tissue specimen from cholesterol group with H&E at LM X 10. CV = central vein

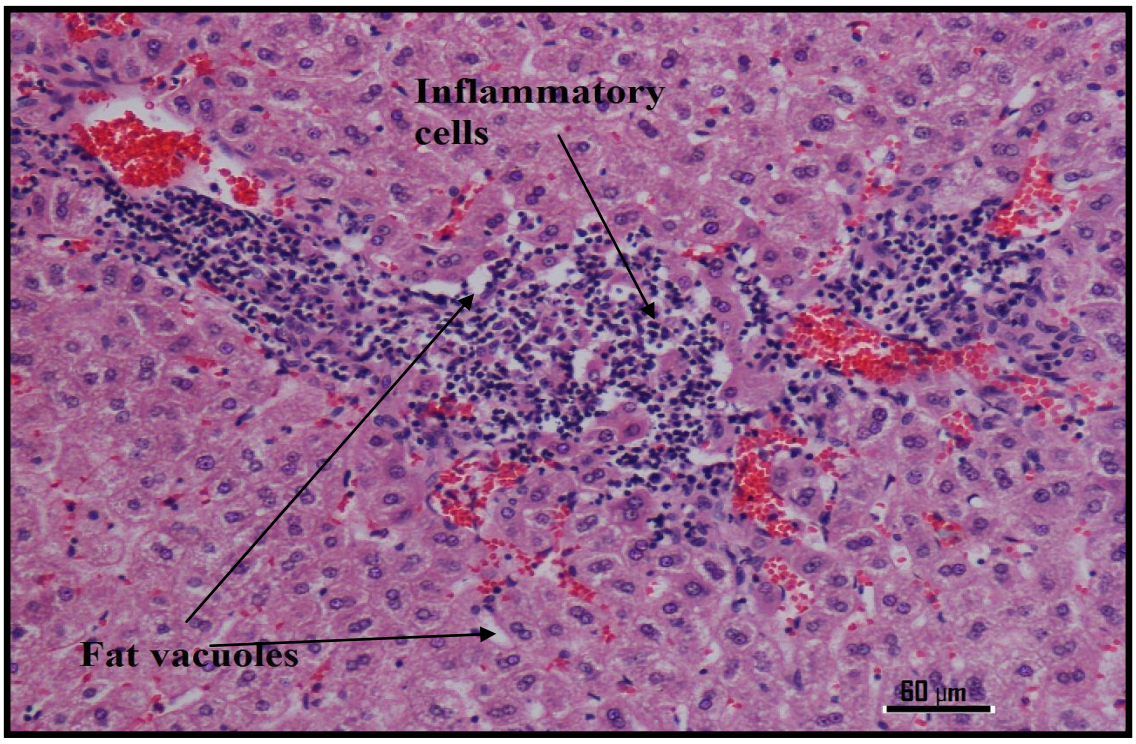


Figure 4.11.20: Cross section of liver tissue specimen from cholesterol group with H&E at LM X 20.

***R. tomentosa* group**

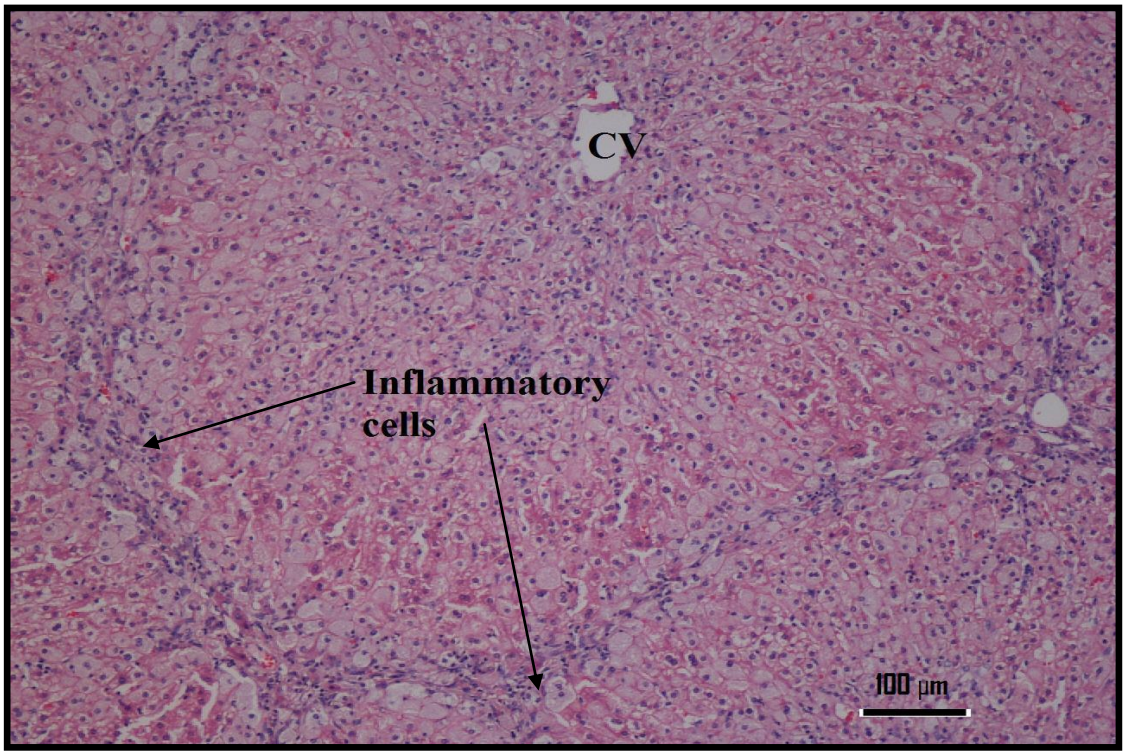


Figure 4.11.21: Cross section of liver tissue specimen from *R. tomentosa* group with H&E at LM X 10.

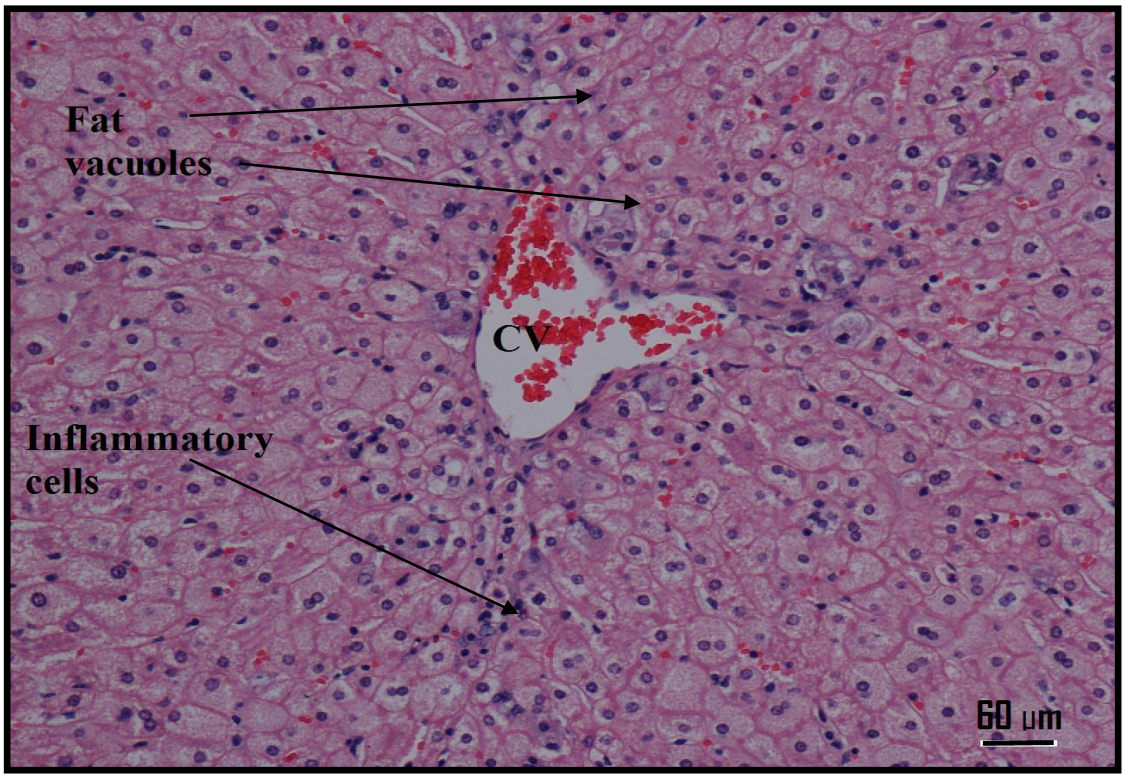


Figure 4.11.22: Cross section of liver tissue specimen from *R. tomentosa* group with H&E at LM X 10.

Simvastatin group

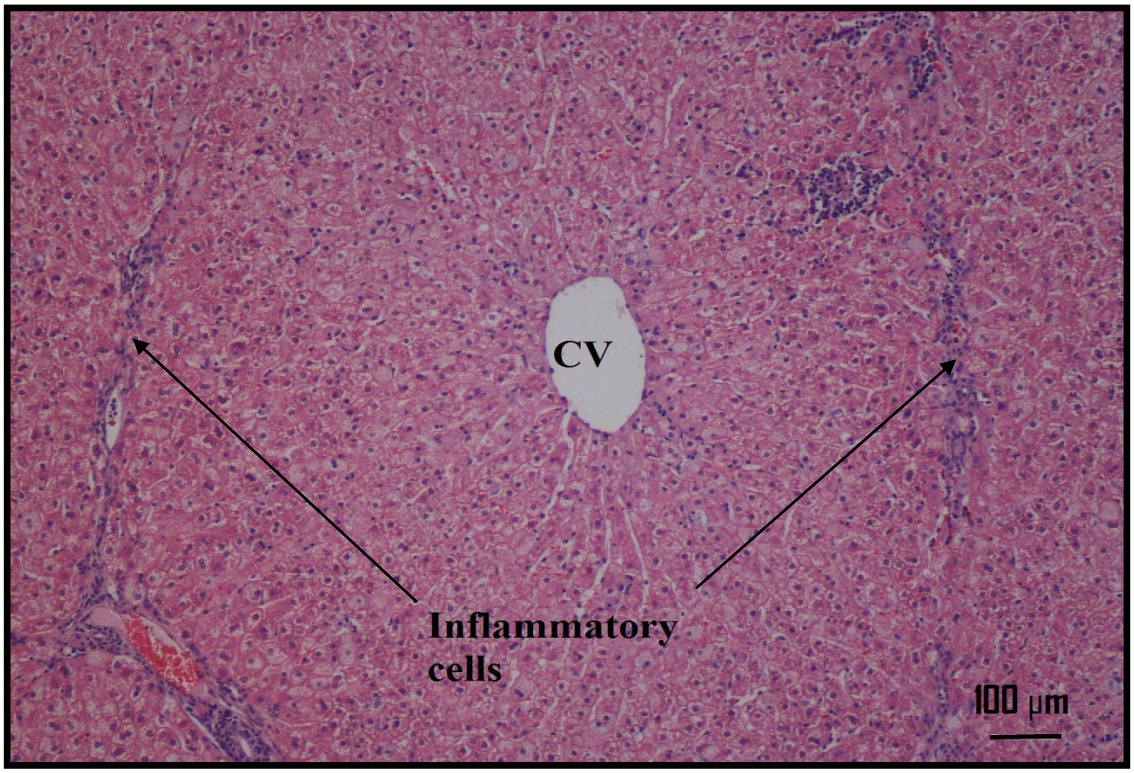


Figure 4.11.23: Cross section of liver tissue specimen from simvastatin group with H&E at LM X 10. CV = central vein

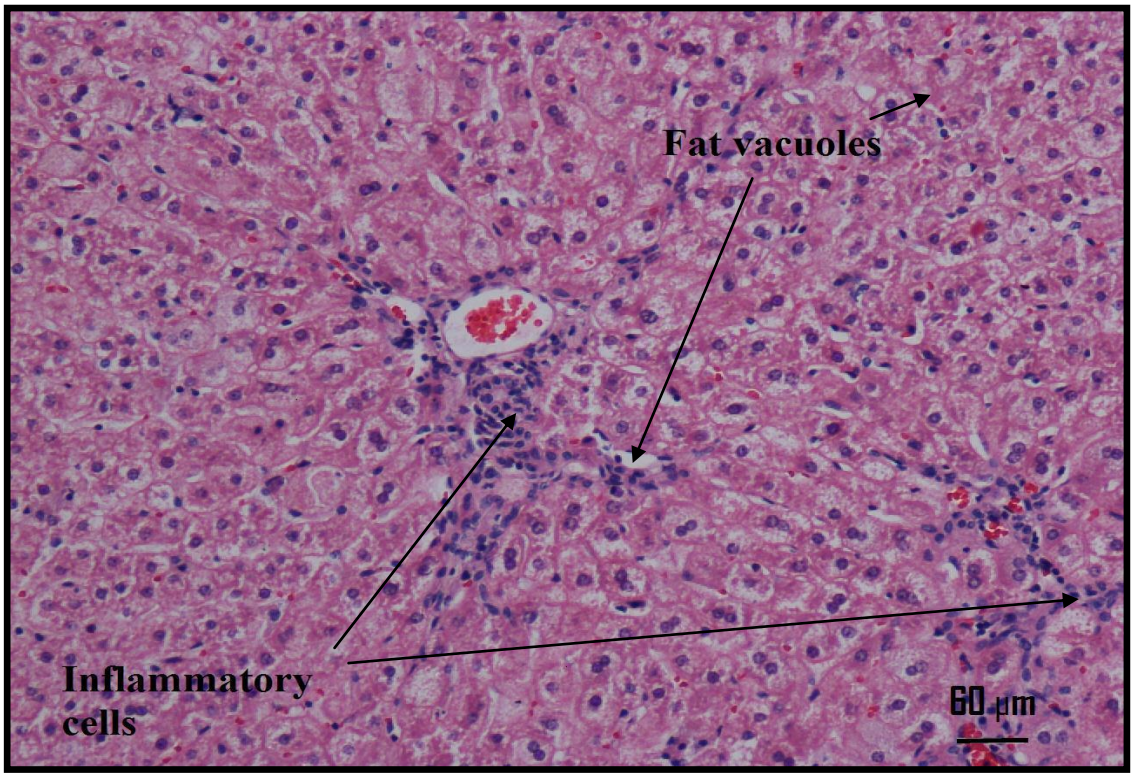


Figure 4.11.24: Cross section of liver tissue specimen from simvastatin group with H&E at LM X 20. CV = central vein

4.12 Evaluation of Atherosclerotic Area Macroscopically.

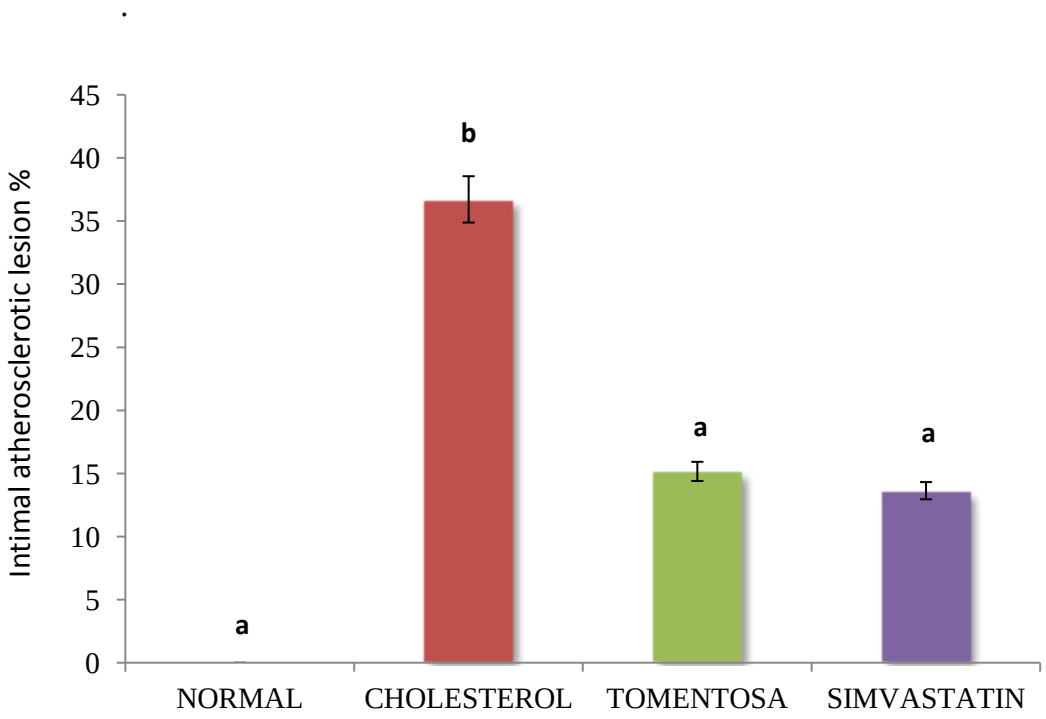


Figure 4.12.1: Histomorphoric analysis of intimal lesion area in different group

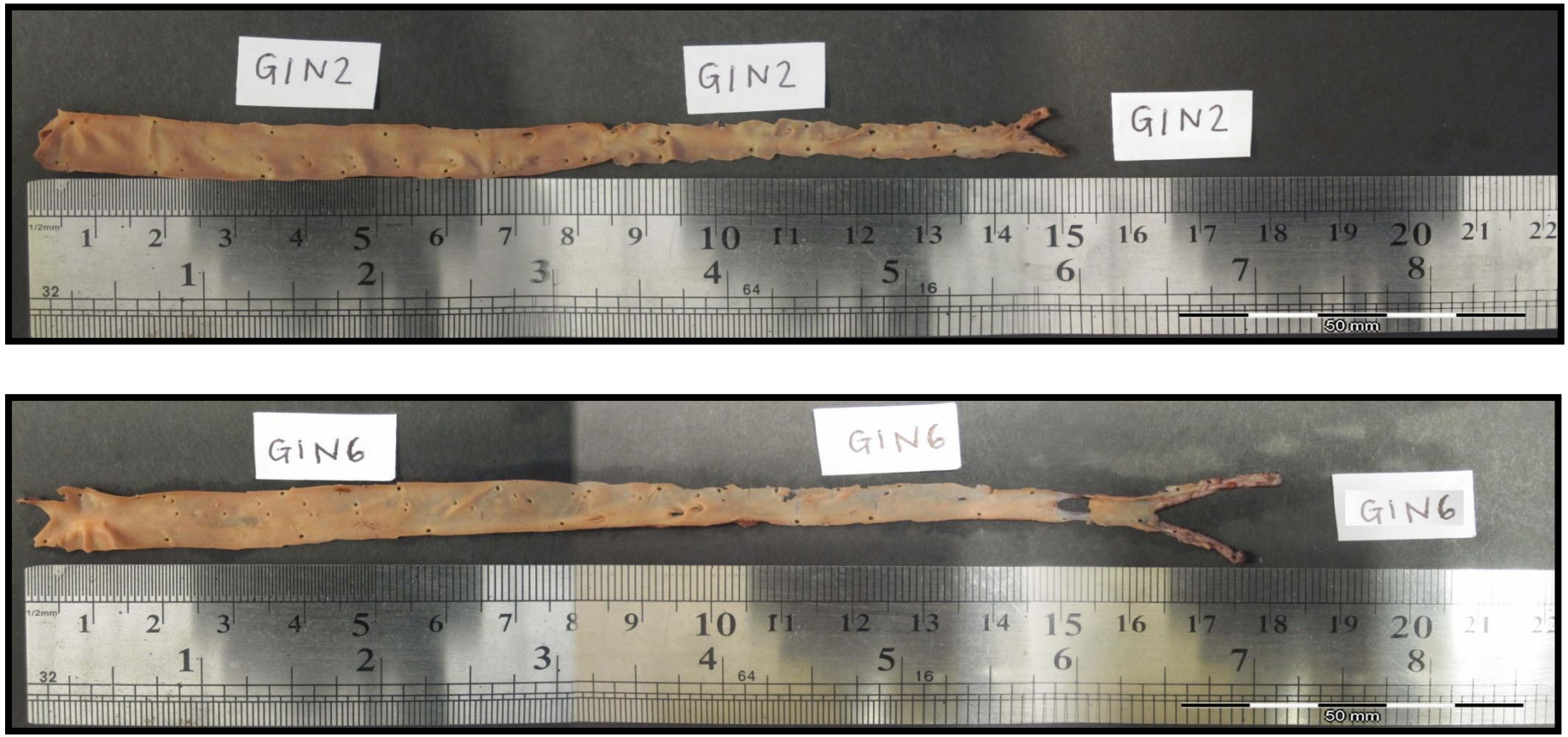


Figure 4.12.2: Aortas of Rabbit in Normal group which stained with Sudan IV without showing any atherosclerotic area

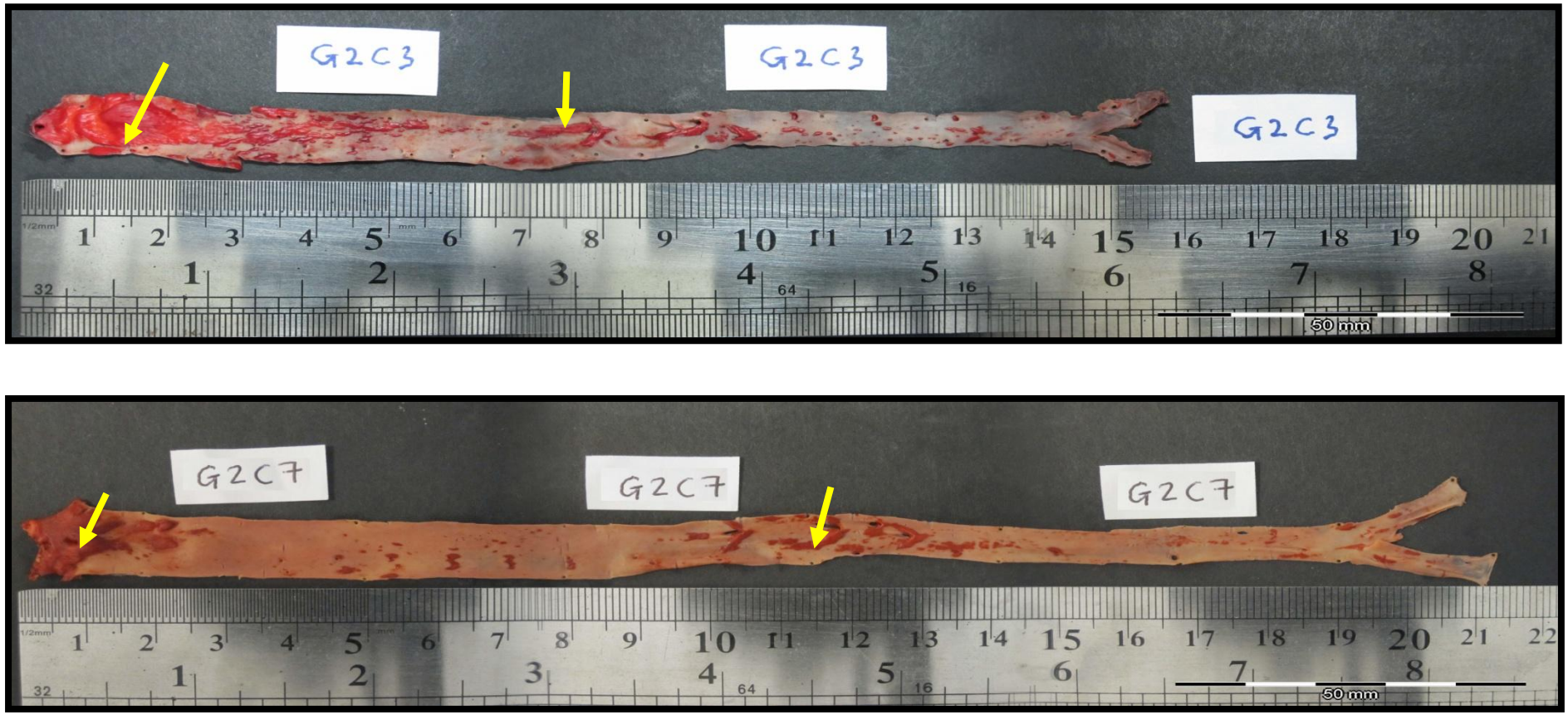


Figure 4.12.3: Aortas of Rabbit in Cholesterol group which stained with Sudan IV showing marked atherosclerotic area. Yellow arrows indicate the presence of atherotoma

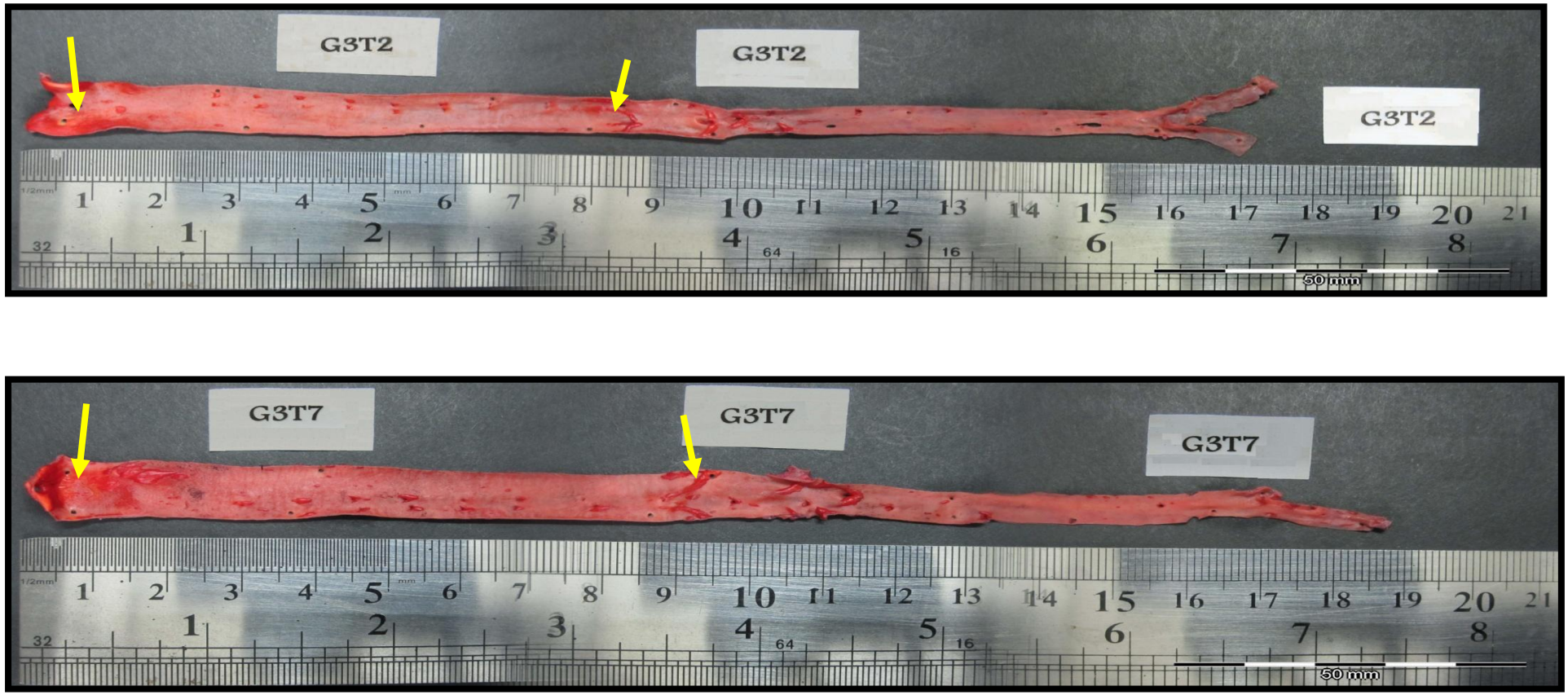


Figure 4.12.4: Aortas of Rabbit in Tomentosa group which stained with Sudan IV showing atherosclerotic area. Yellow arrows indicate the presence of atherotoma

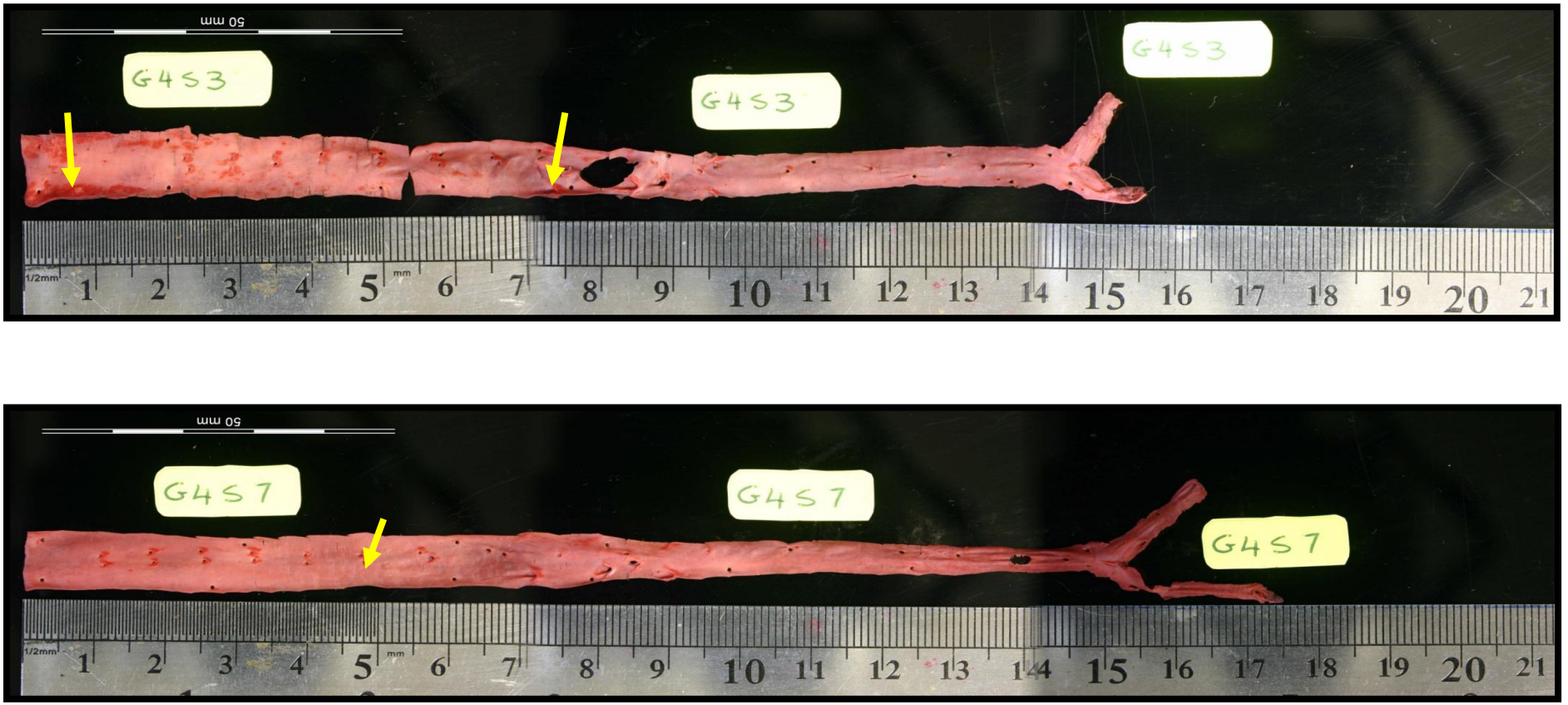


Figure 4.12.5: Aortas of Rabbit in Simvastatin group which stained with Sudan IV showing atherosclerotic area. Yellow arrows indicate the presence of atherotoma

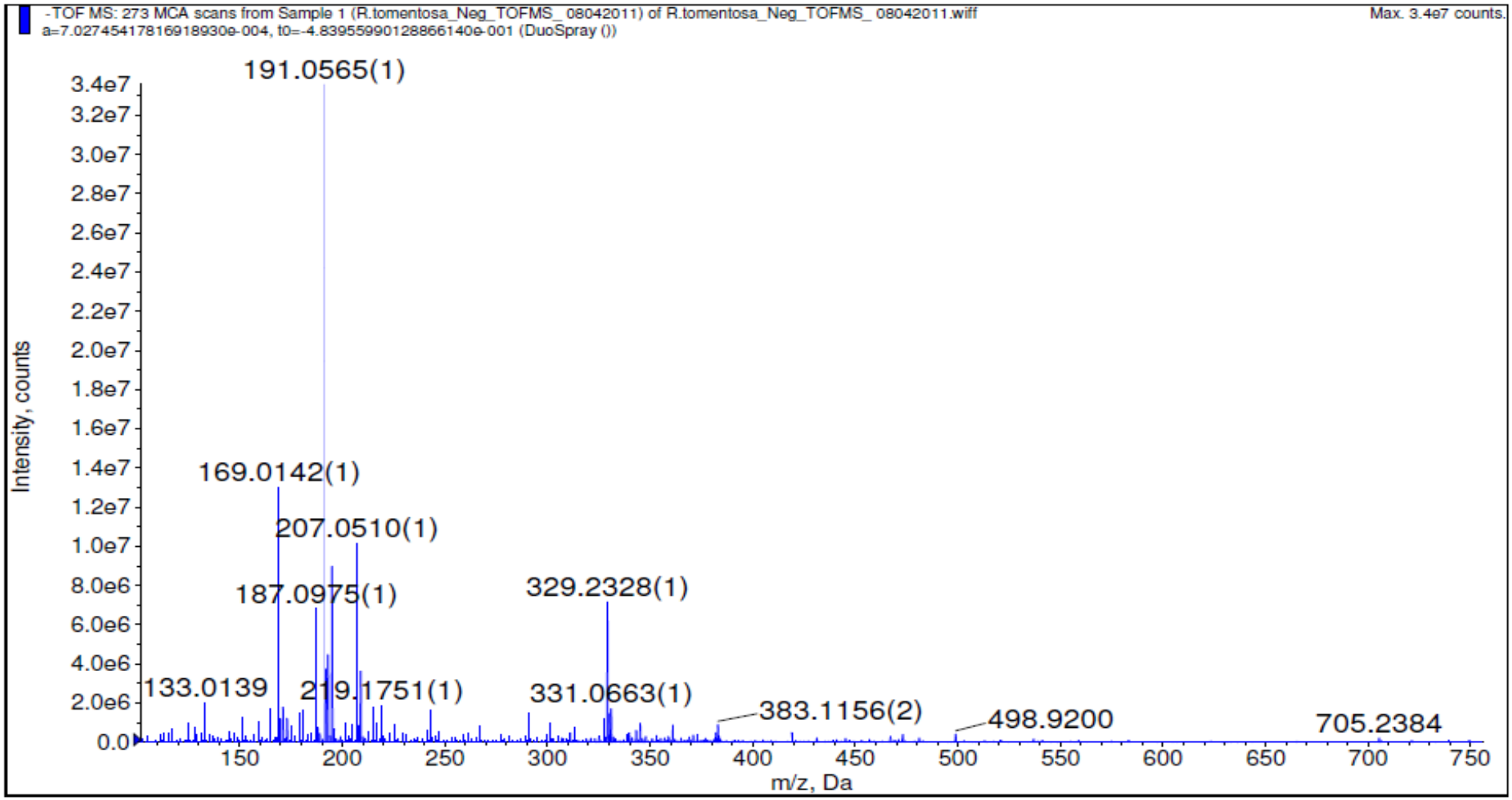


Figure 4.2.13: ESI-MS at scan mode of water extract of *R. tomentosa* fruit TOF-MS 100-750

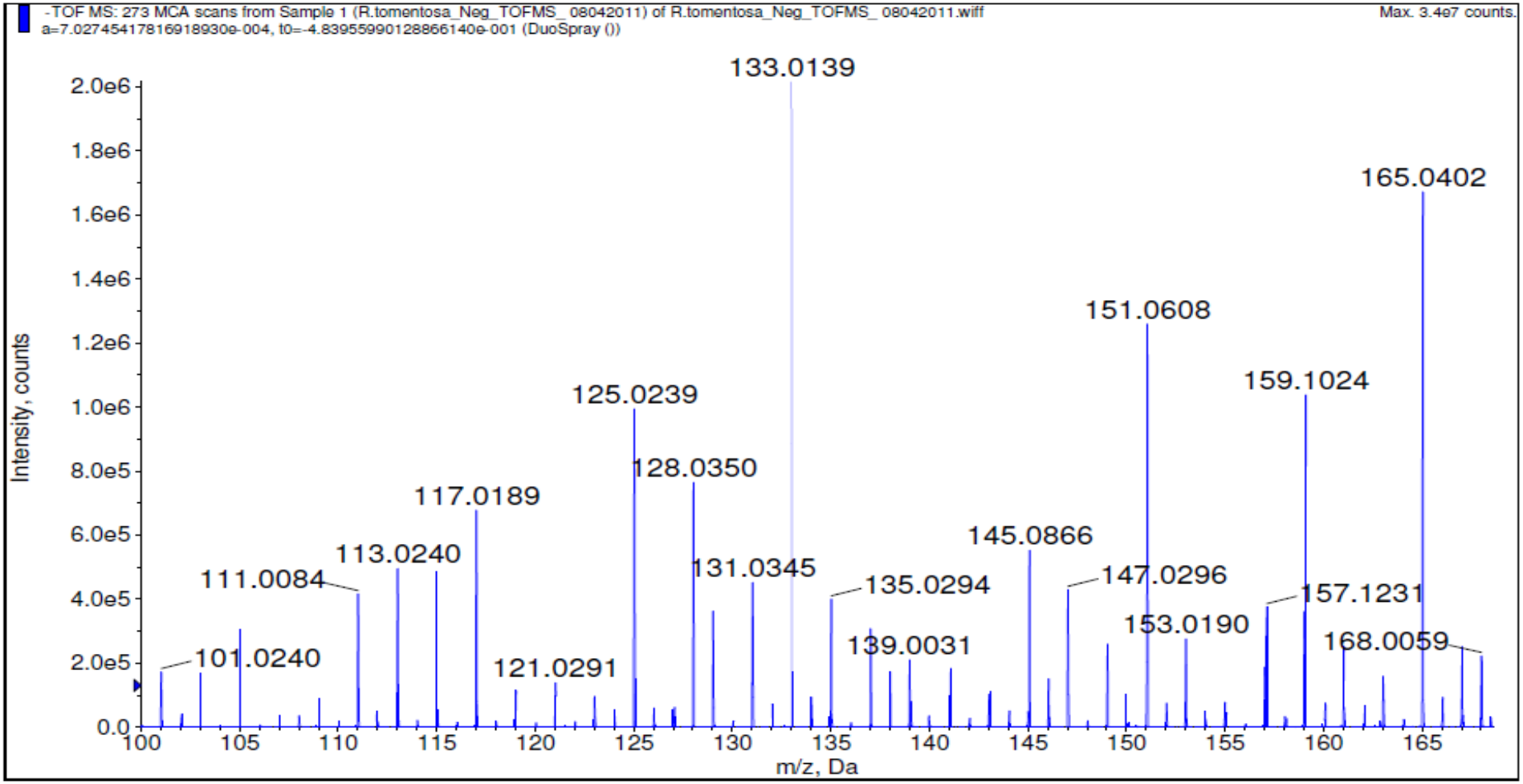


Figure 4.2.14: ESI-MS at scan mode of water extract of *R. tomentosa* fruit TOF-MS 100-165

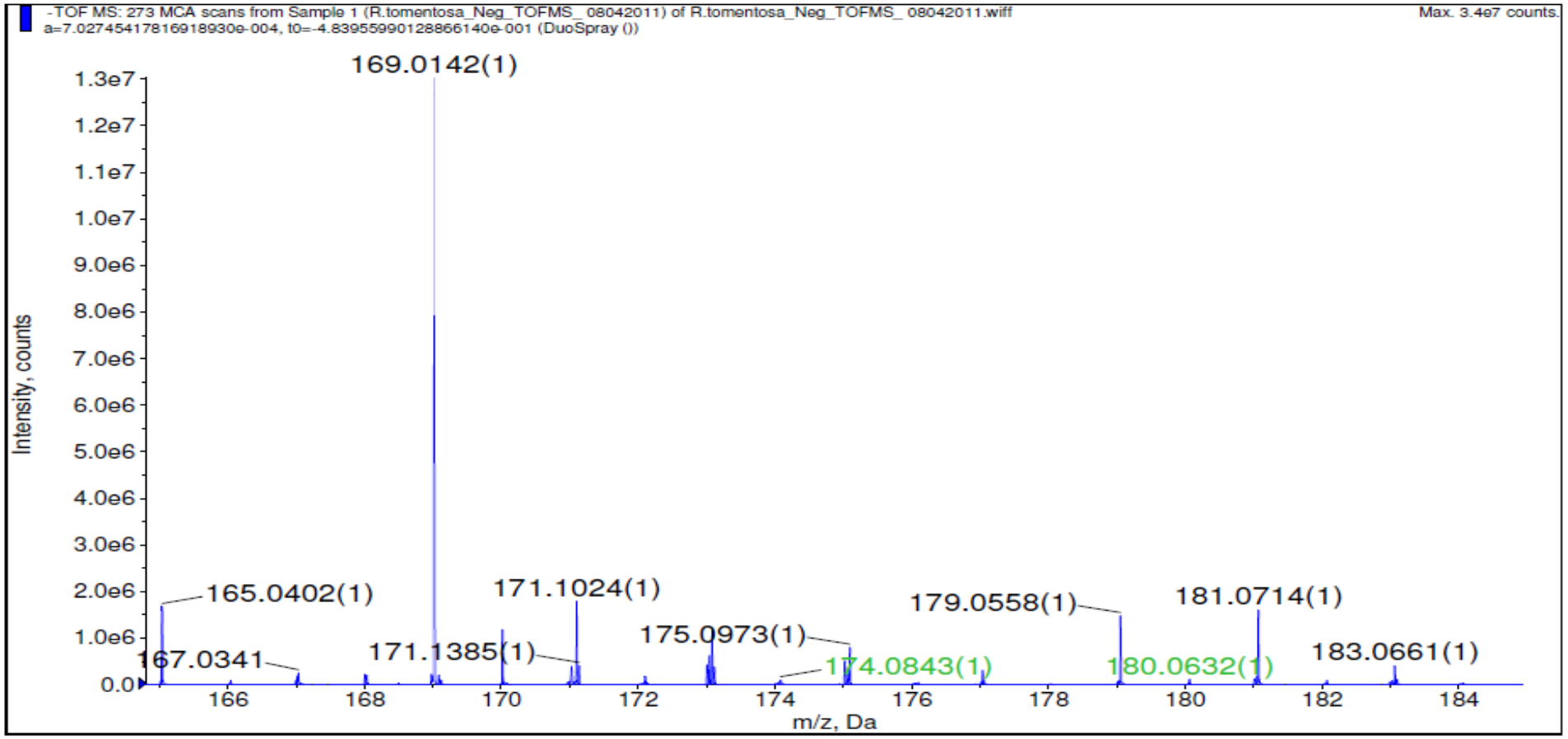


Figure 4.2.15: ESI-MS at scan mode of water extract of *R. tomentosa* fruit TOF-MS 165-184

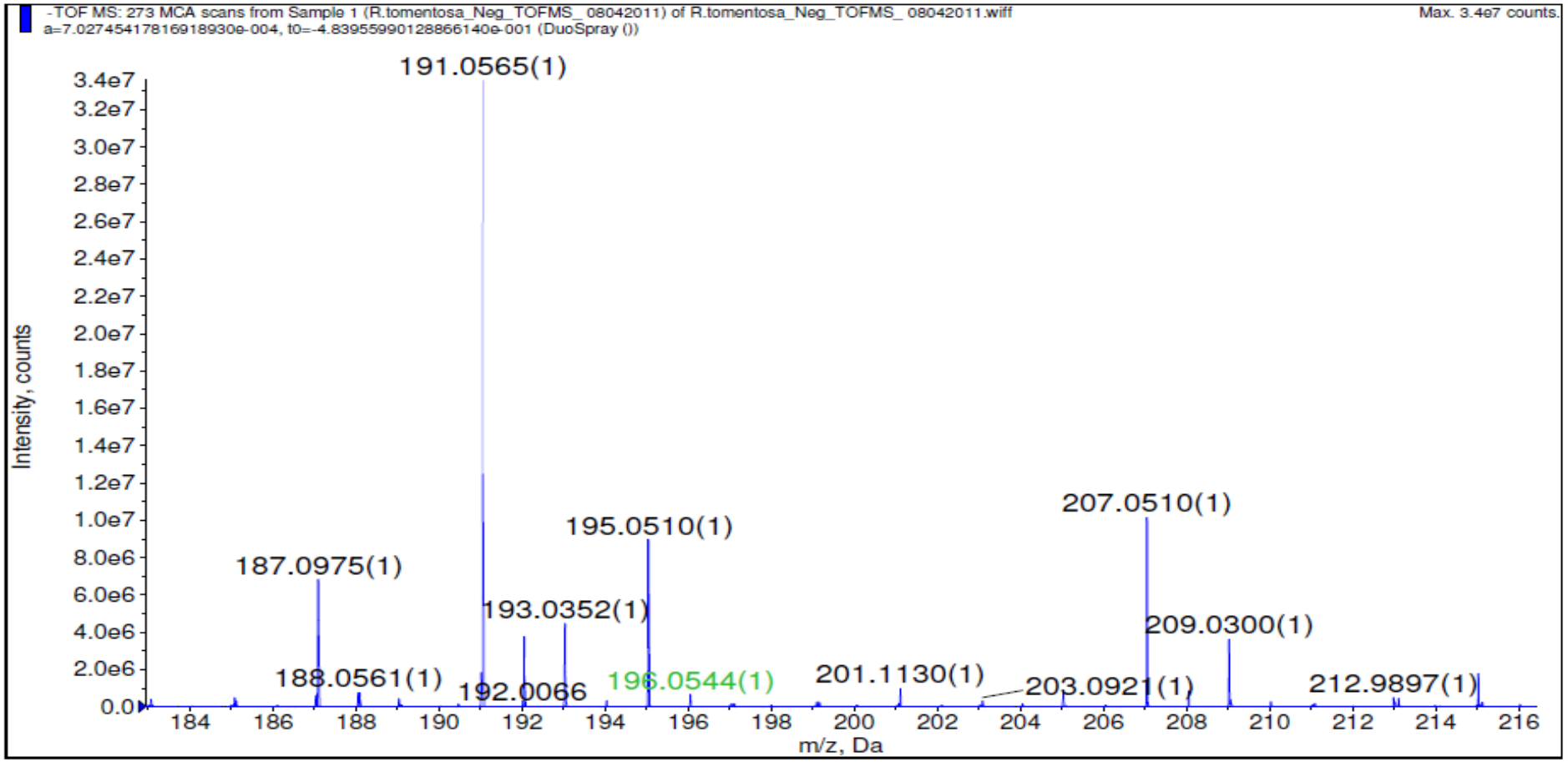


Figure 4.2.16: ESI-MS at scan mode of water extract of *R. tomentosa* fruit TOF-MS 184-215

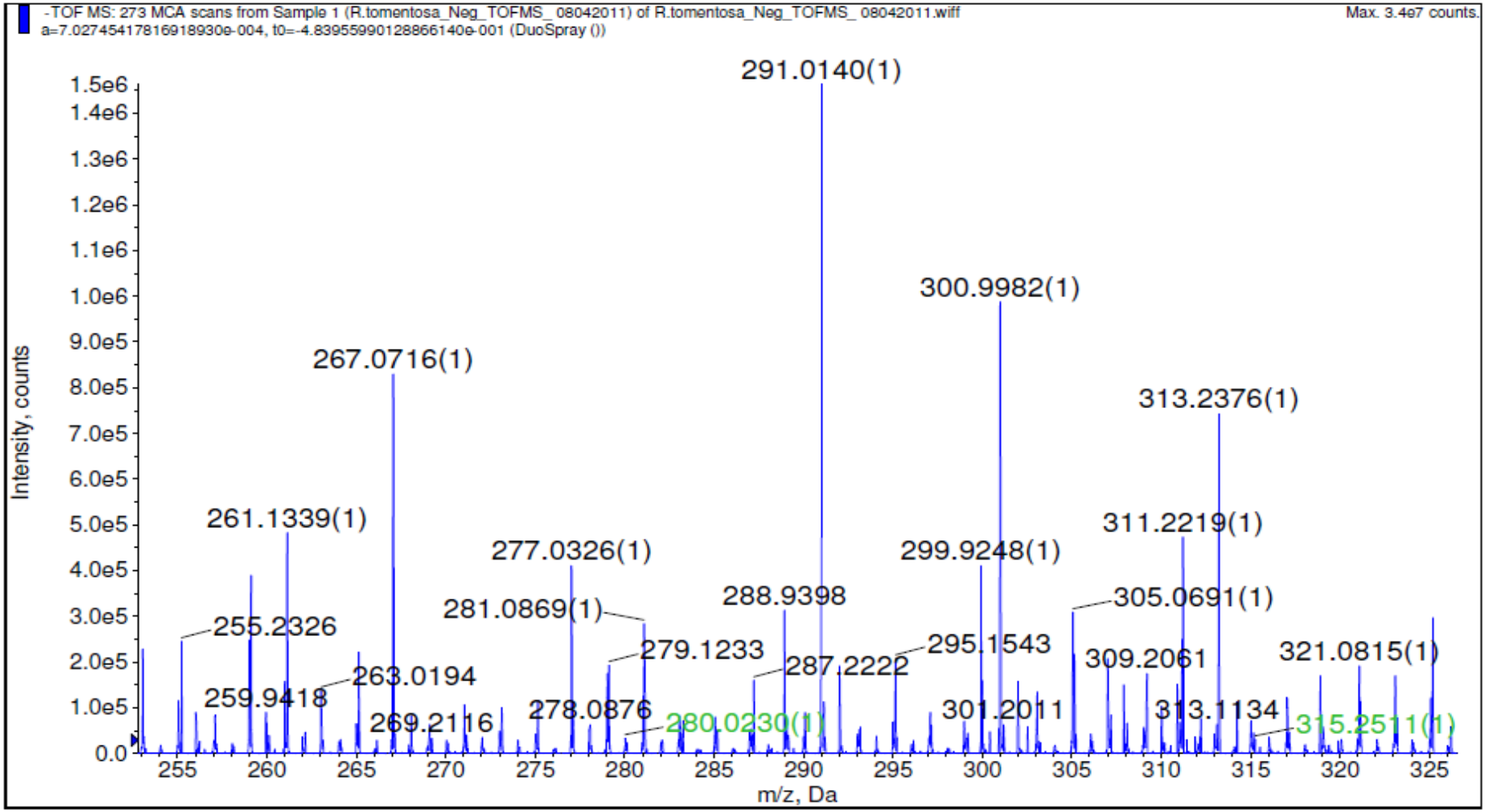


Figure 4.2.17: ESI-MS at scan mode of water extract of *R. tomentosa* fruit TOF-MS 255-325

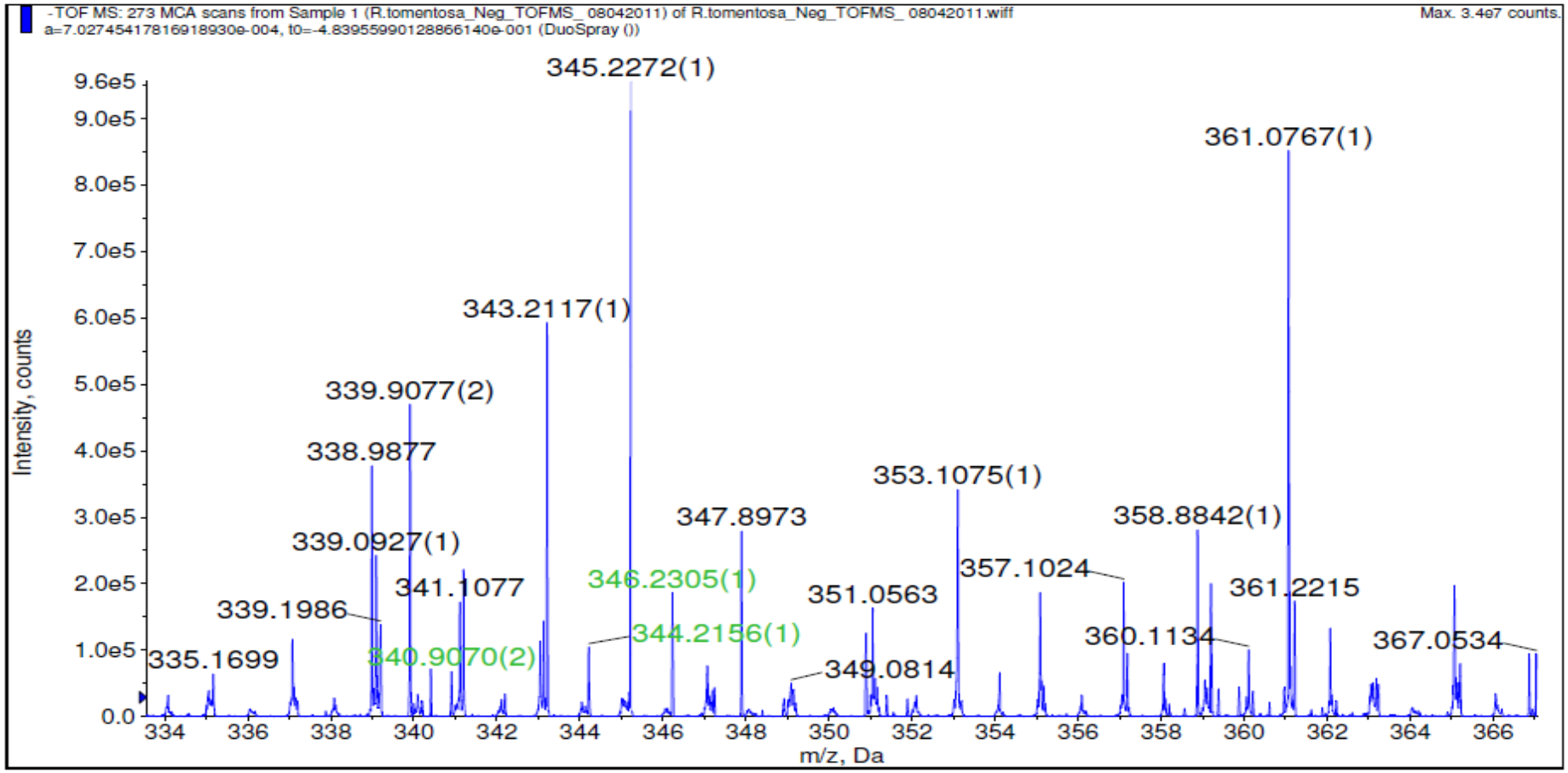


Figure 4.2.18: ESI-MS at scan mode of water extract of *R. tomentosa* fruit TOF-MS 334-365

Table 4.2.3: Identification of phytochemicals in water extracts from *R. tomentosa* fruit extract

No	$[M+H]^+$ (m/z)	Experimental MS^2 (m/z)	Compound identified
1	133.0139	115, 89, 73, 71, 59	Malic acid
2	151.0658	136, 132, 119, 107, 101, 89, 71, 59	Unidentified
3	165.0402	147, 129, 75, 59	Unidentified
4	169.0142	151, 125, 108	Gallic acid
5	170.0173	126, 125, 108	Unidentified
6	171.1024	153,139, 127, 125, 111, 109, 108	Unidentified
7	179.0558	161, 135, 134, 99, 87, 75, 71, 59	Caffeic acid
8	181.0714	166, 163, 151, 137, 135,119, 109, 101	Dihydrocaffeic acid
9	187.0975	169, 143, 125, 123, 97, 79, 57	Unidentified
10	191.0565	171, 137, 127, 109, 93, 87, 85, 81, 67, 59	Quinic acid
11	192.0597	Low intensity	Unidentified
12	193.0352	175, 149, 134, 129, 115, 113, 103, 89, 85, 72, 71, 59	Unidentified
13	195.0510	177, 159, 150, 129, 111, 99, 87,75, 59	Unidentified
14	201.1130	199, 183, 171, 164, 157, 139, 137, 127, 111	Unidentified
15	207.0510	147, 129, 109, 85, 72	Unidentified
16	208.0542	Low intensity	Unidentified
17	209.0300	191, 165, 147, 141, 133, 129, 115, 111, 101, 89, 85, 71, 59	Unidentified
18	225.1128	207, 166, 137, 121, 109, 96, 75, 59	Unidentified
19	243.1234	225, 207, 201, 199, 181, 163, 124, 110, 99, 71,59	Unidentified
20	267.0716	249, 223, 207, 191, 181, 169, 125, 113	Unidentified
21	291.0140	247, 219, 203, 191, 175, 171, 125, 80	Brevifolin carboxylic acid
22	301.0082	Low intensity	Unidentified
23	313.2376	277, 251, 201, 191, 183, 171, 127	Unidentified

Table 4.2.3 Continued

No	$[M+H]^+$ (m/z)	Experimental MS^2 (m/z)	Compound identified
24	327.2169	Low intensity	Unidentified
25	329.2328	311, 293, 270, 229, 211, 201, 183, 171, 139, 127, 99	Octadecenoic acid
25	331.0663	313, 295, 271, 239, 211, 169,168, 125, 124, 107, 89	Galloyl glucose

4.2 HPLC and GCMS analyses of *R. tomentosa* extract

Table 4.2.1: Retention time of standard for HPLC analysis.

Standard	Retention time at 280nm	Retention time at 360nm
Gallic acid	4.938	4.941
Quercetine	31.942	32.506
Tannic acid	4.981	4.968

Table 4.2.2: Retention time of *R. tomentosa* extract for HPLC analysis.

Standard	Retention time at 280nm	Retention time at 360nm
Gallic acid	4.924	4.941
Quercetine	31.830	32.475
Tannic acid	4.955	4.945

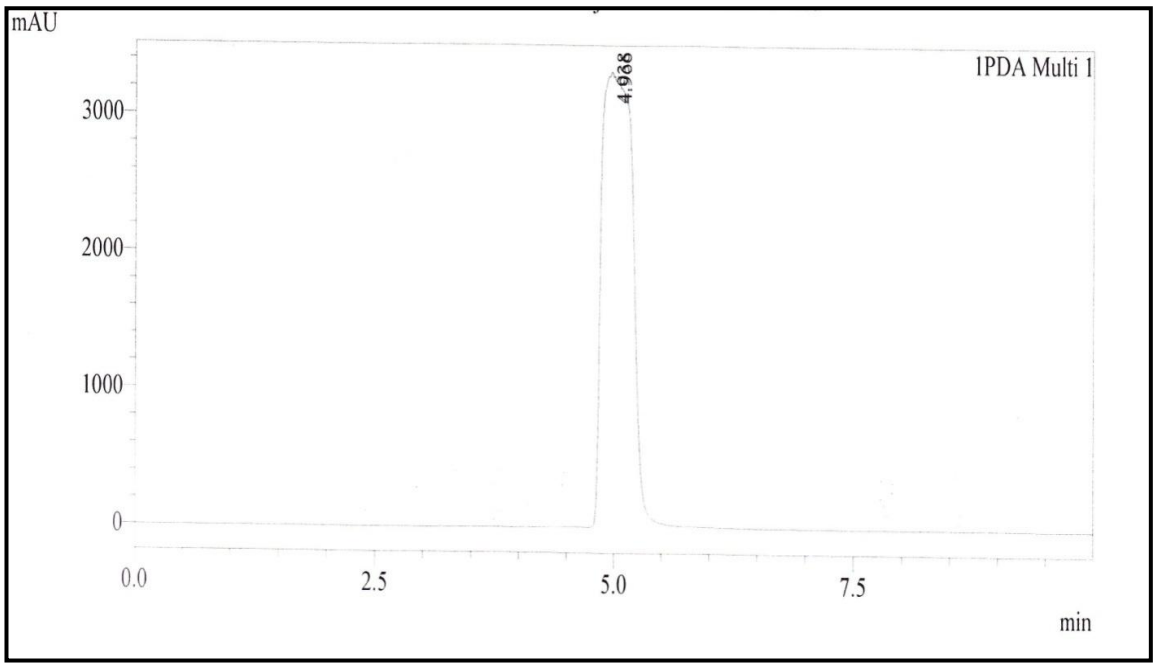


Figure 4.2.1: HPLC chromatogram of standard gallic acid at wavelength 280nm. Peak was marked with its retention time.

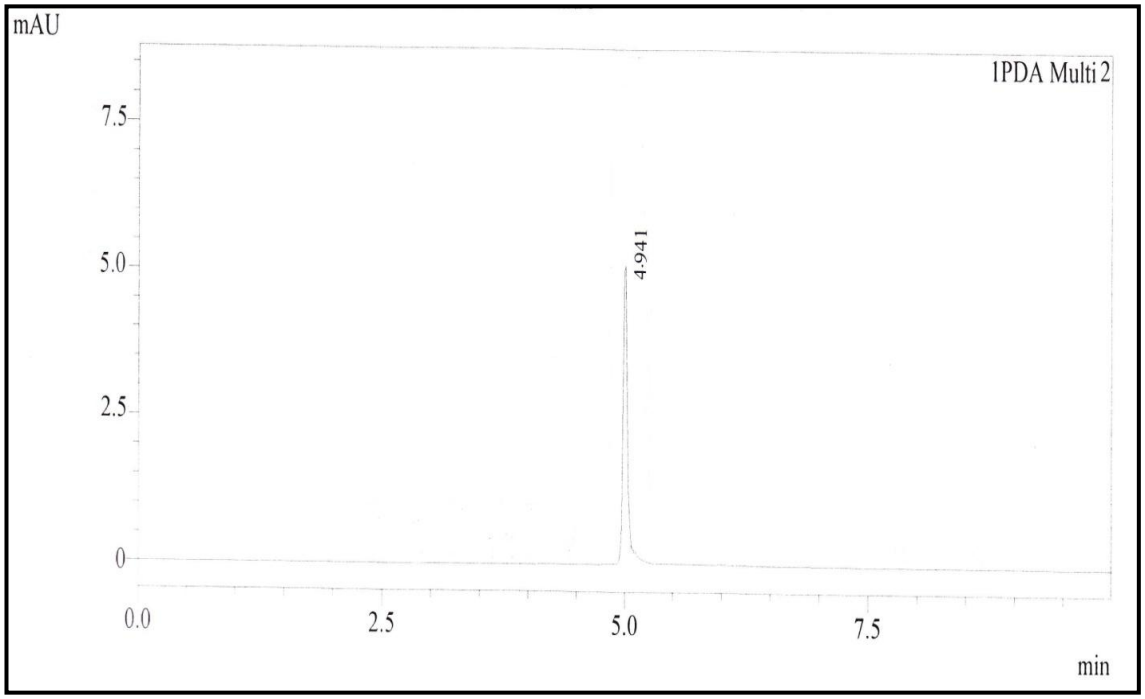


Figure 4.2.2: HPLC chromatogram of standard gallic acid at wavelength 360nm. Peak was marked with its retention time.

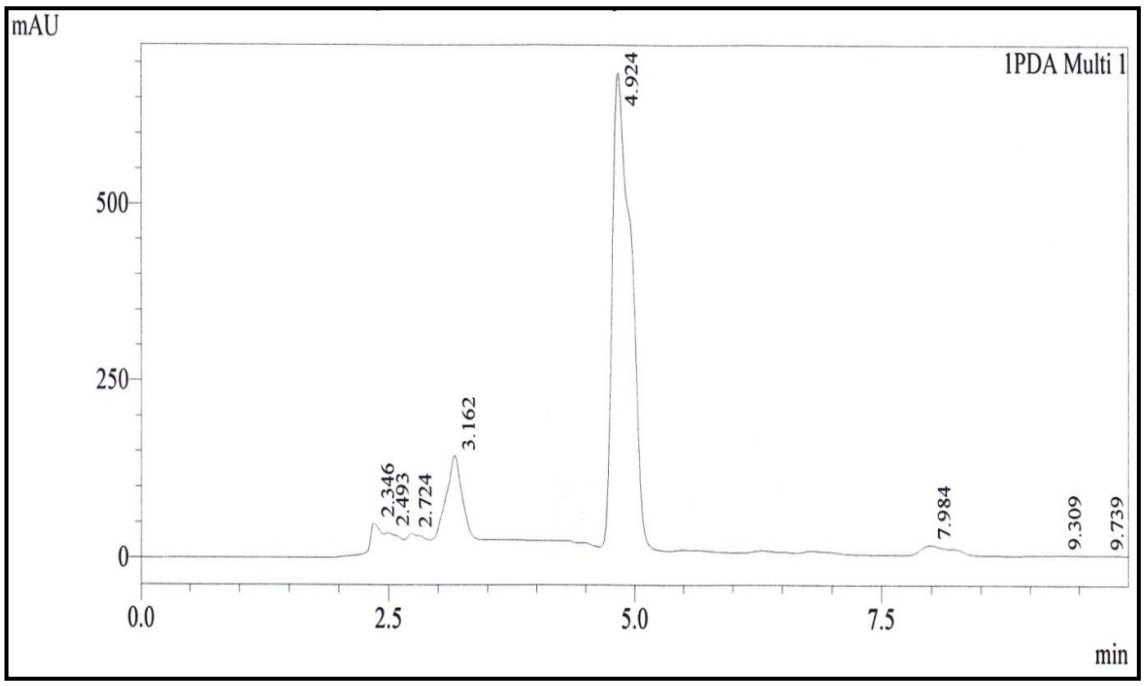


Figure 4.2.3: HPLC chromatogram of *R. tomentosa* at wavelength 280nm. Peak was marked with its retention time.

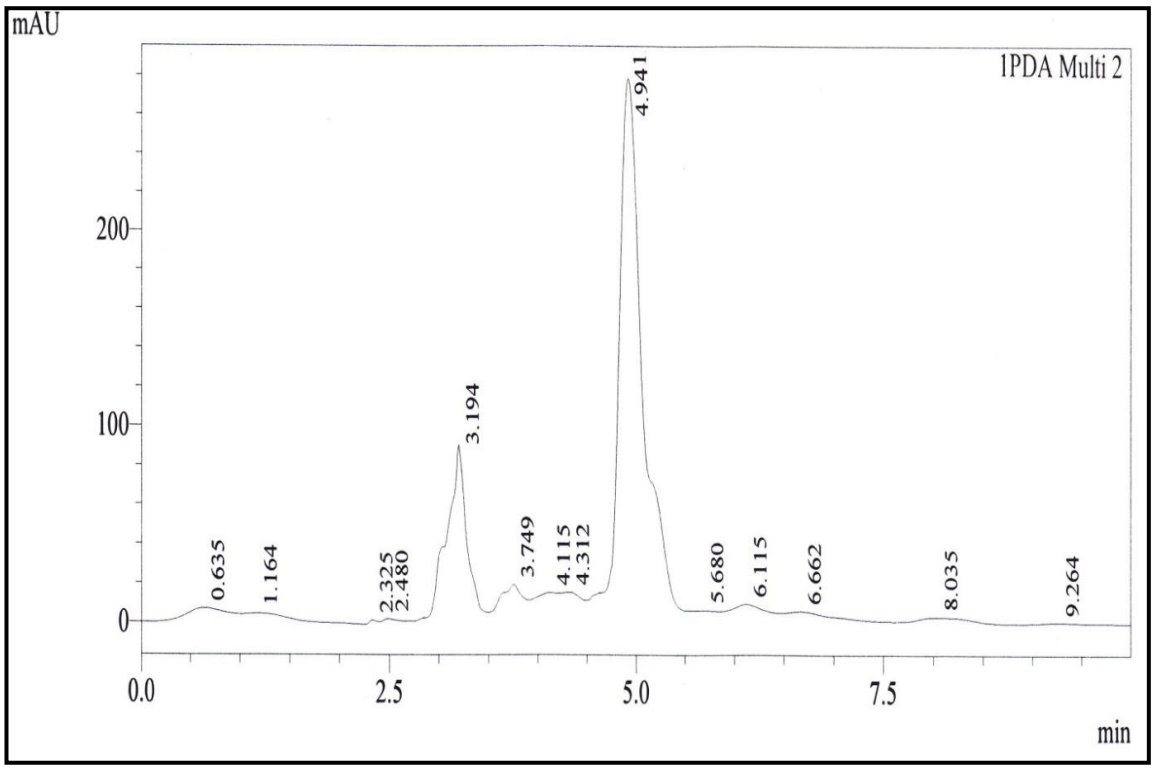


Figure 4.2.4: HPLC chromatogram of *R. tomentosa* wavelength 360nm. Peak was marked with its retention time.

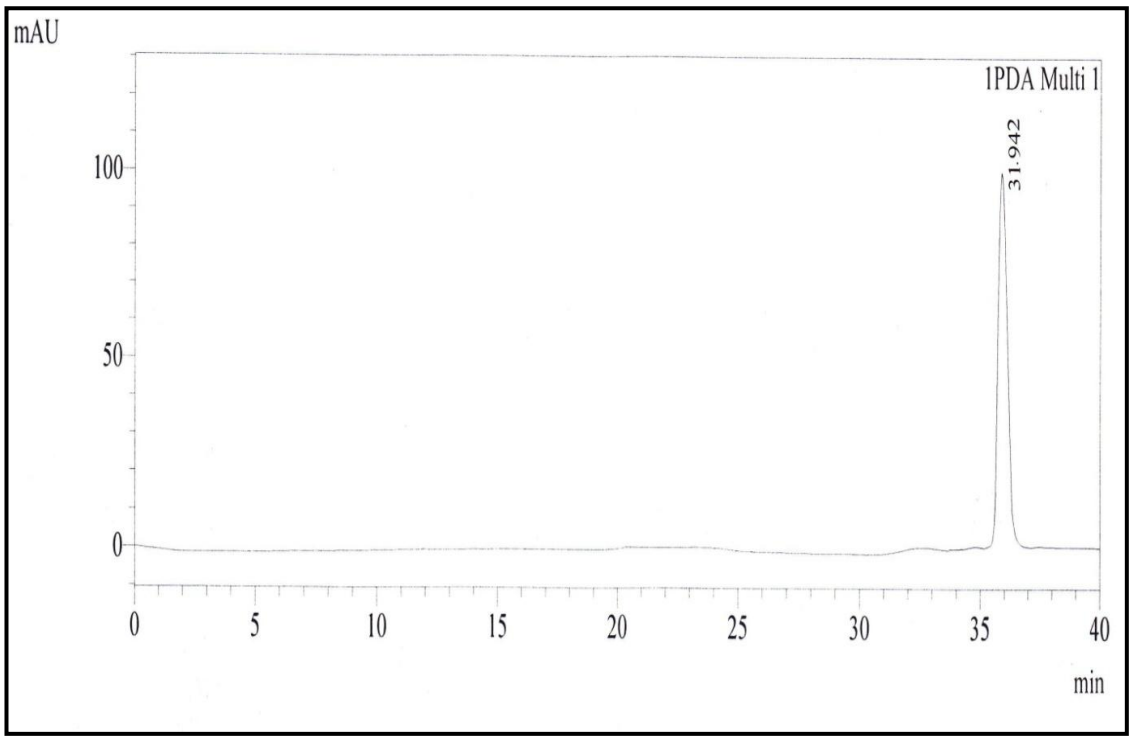


Figure 4.2.5: HPLC chromatogram of standard quercetin at wavelength 280nm. Peak was marked with its retention time.

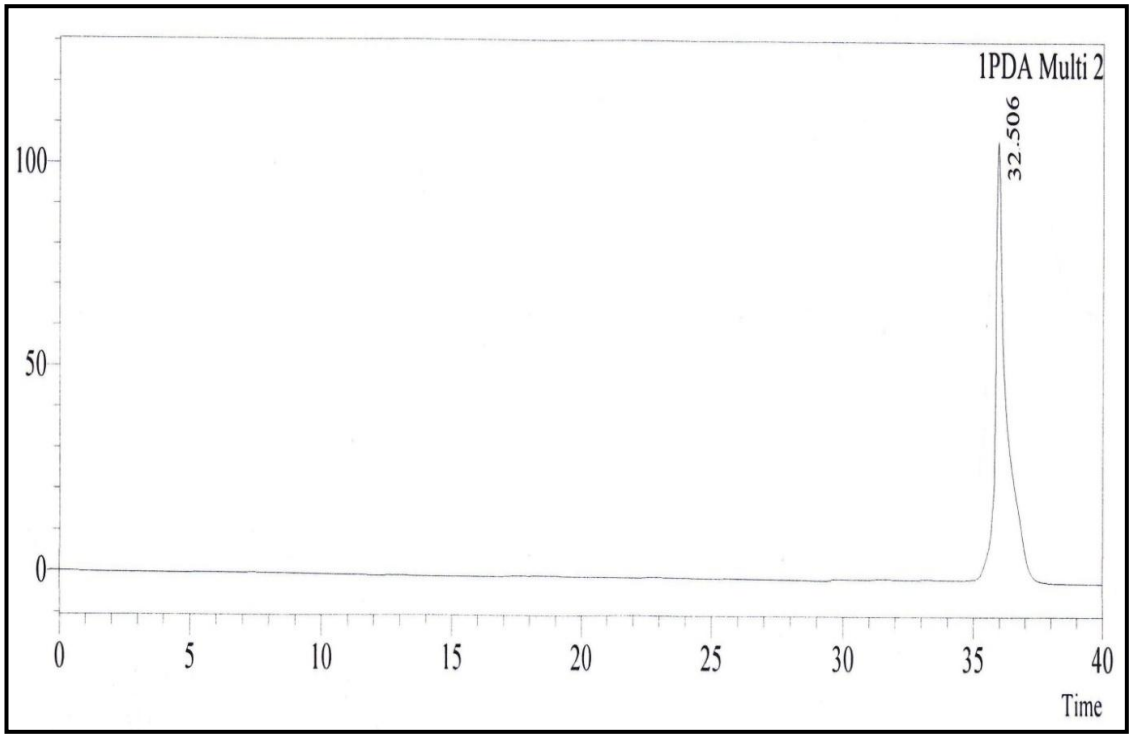


Figure 4.2.6: HPLC chromatogram of standard quercetin at wavelength 360nm. Peak was marked with its retention time.

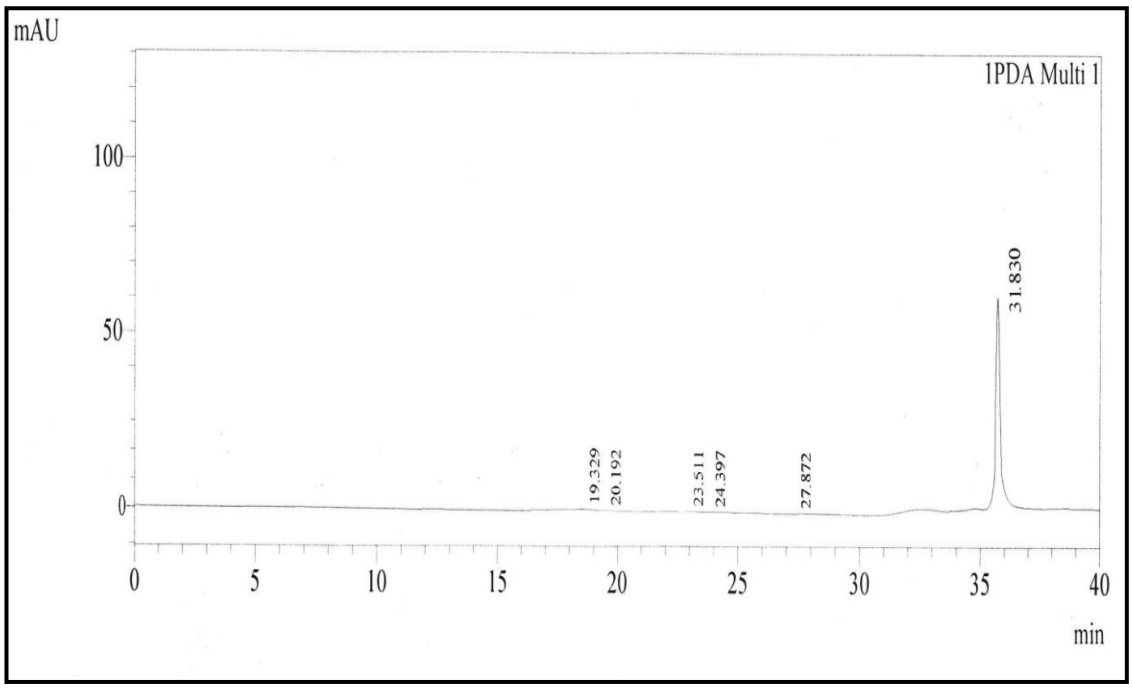


Figure 4.2.7: HPLC chromatogram of *R. tomentosa* at wavelength 280nm. Peak was marked with its retention time.

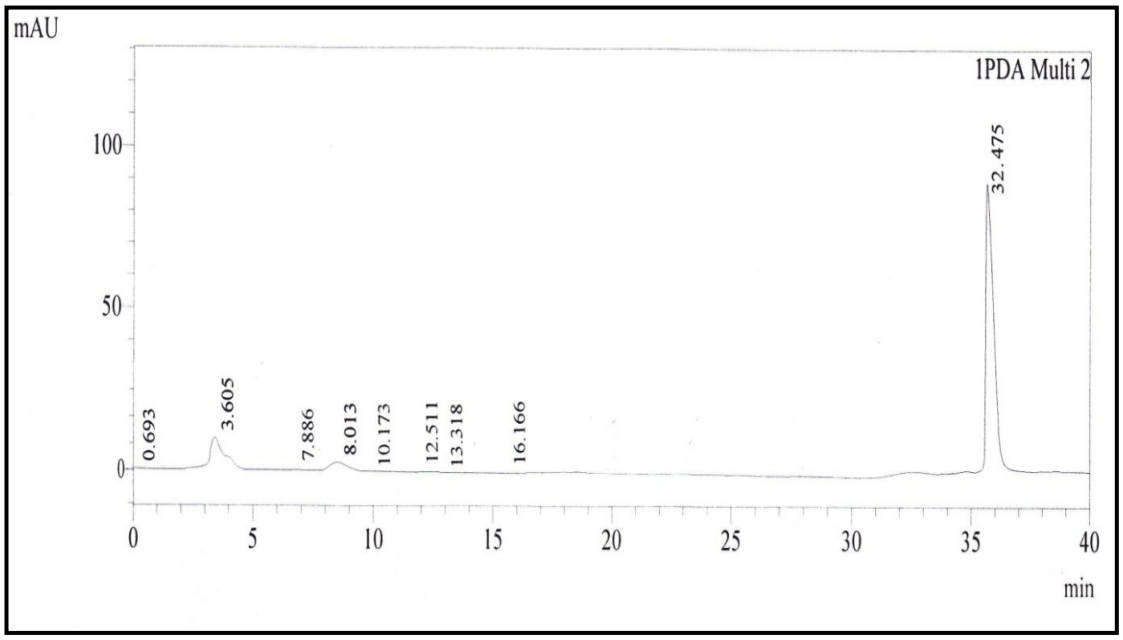


Figure 4.2.8: HPLC chromatogram of *R. tomentosa* at wavelength 360nm. Peak was marked with its retention time.

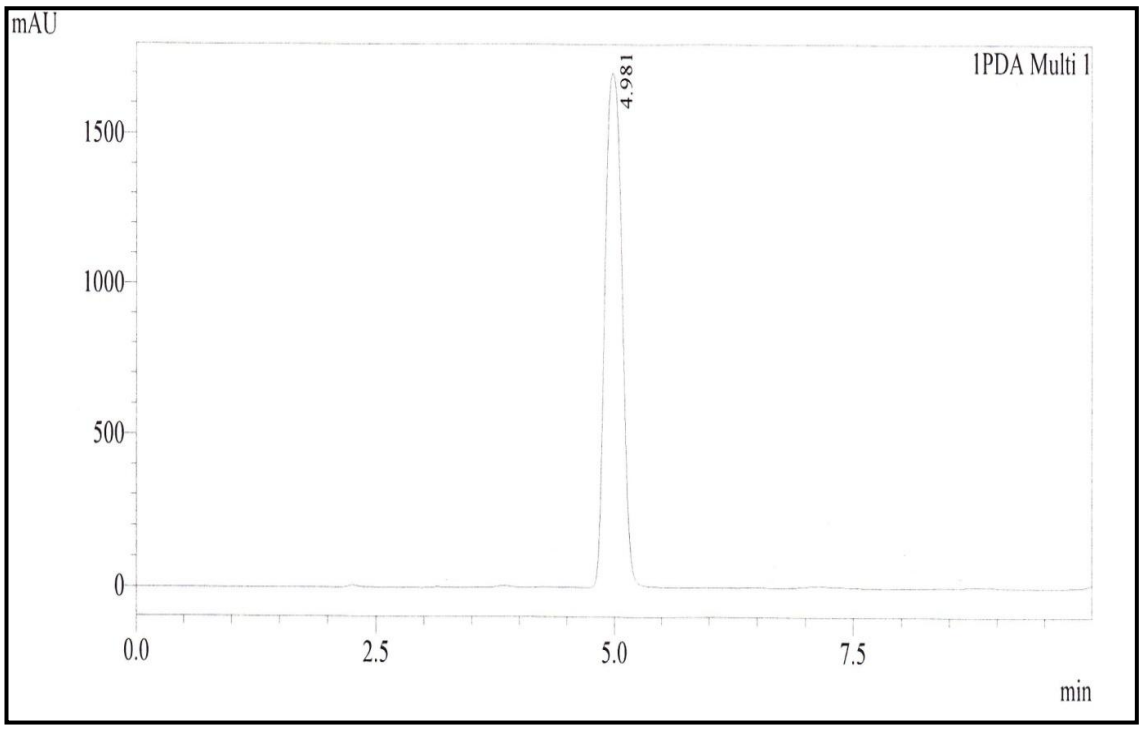


Figure 4.2.9: HPLC chromatogram of standard tannic acid at wavelength 280nm. Peak was marked with its retention time.

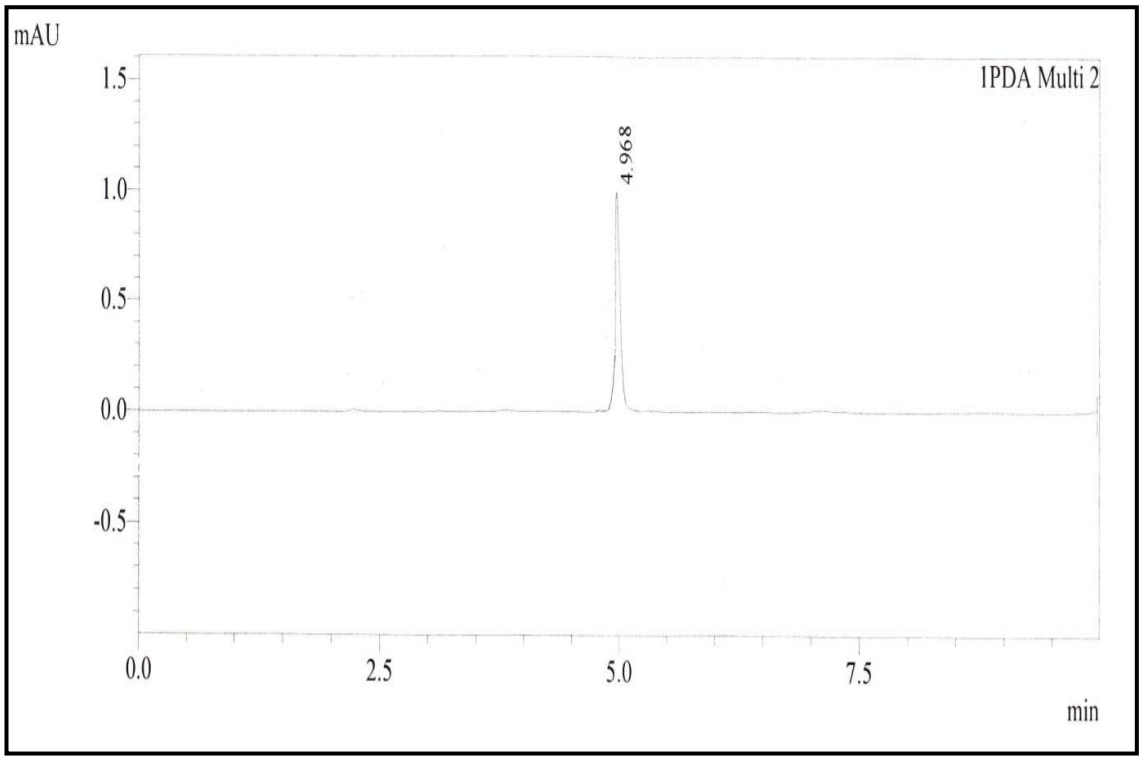


Figure 4.2.10: HPLC chromatogram of standard tannic acid at wavelength 360nm. Peak was marked with its retention time.

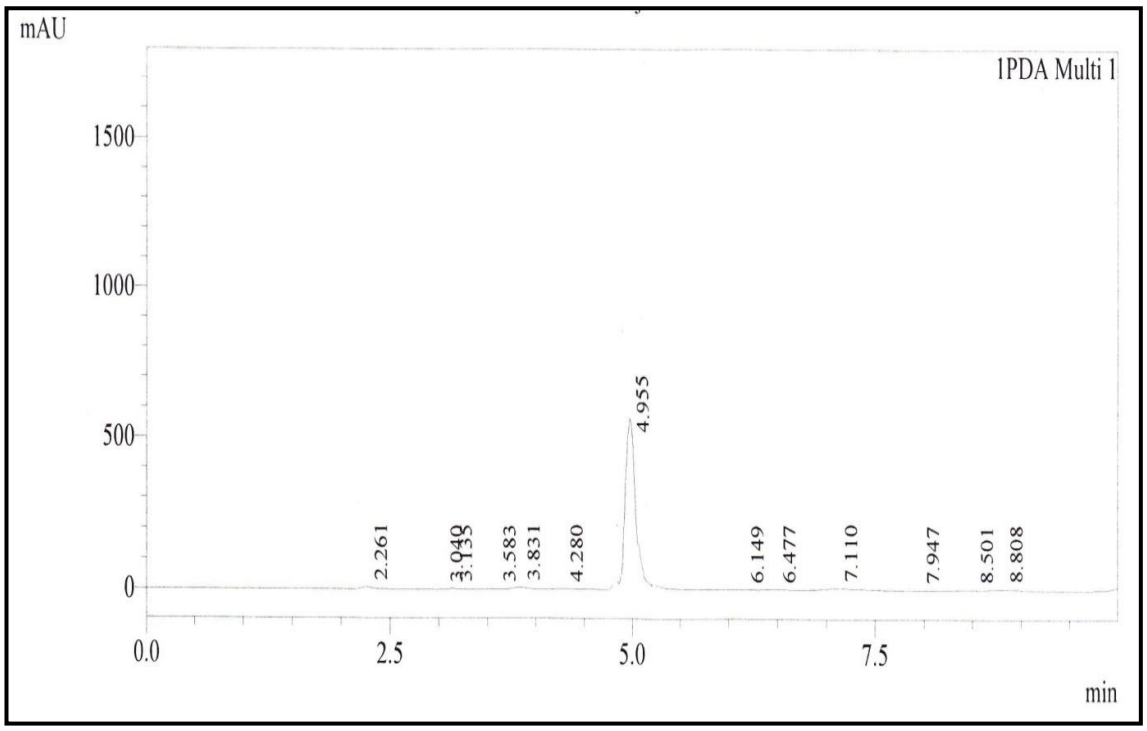


Figure 4.2.11: HPLC chromatogram of *R. tomentosa* at wavelength 280nm. Peak was marked with its retention time.

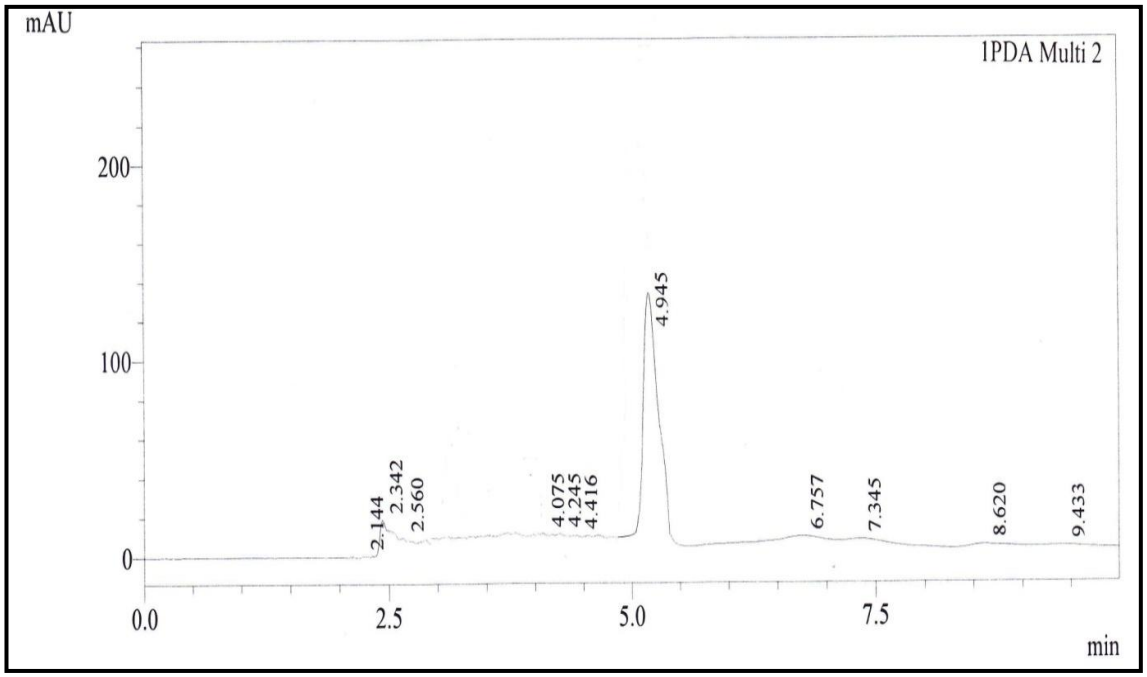


Figure 4.2.12: HPLC chromatogram of *R. tomentosa* at wavelength 360nm. Peak was marked with its retention time.

4.4 Determination of Total Phenolic Contents (TPC)

Table 4.4.1: Total phenolic contents of *Rhodomyrtus tomentosa* extracts. Data presented are in mean ± standard error (n = 3)

Extracts	Total Phenolic Content (mg/g dry mass)
Water	66.515 ± 0.009
Methanol	40.000 ± 0.003
Chloroform	13.985 ± 0.006
Petroleum Ether	12.984 ± 0.002

Total phenol content (TPC) in this current experiment was expressed as gallic acid equivalent. Gallic acid was used as standard and the standard graph had y = 0.0066x and R^2 with 0.9973. Water extract of *R. tomentosa* (65.515 ± 0.009mg/g dry mass) was higher than that on methanol extract (40.000 ± 0.003mg/g drymass). Followed by chloroform extract (13.985 ± 0.006mg/g dry mass) and lastly petroleum ether (12.984 ± 0.002mg/g dry mass).

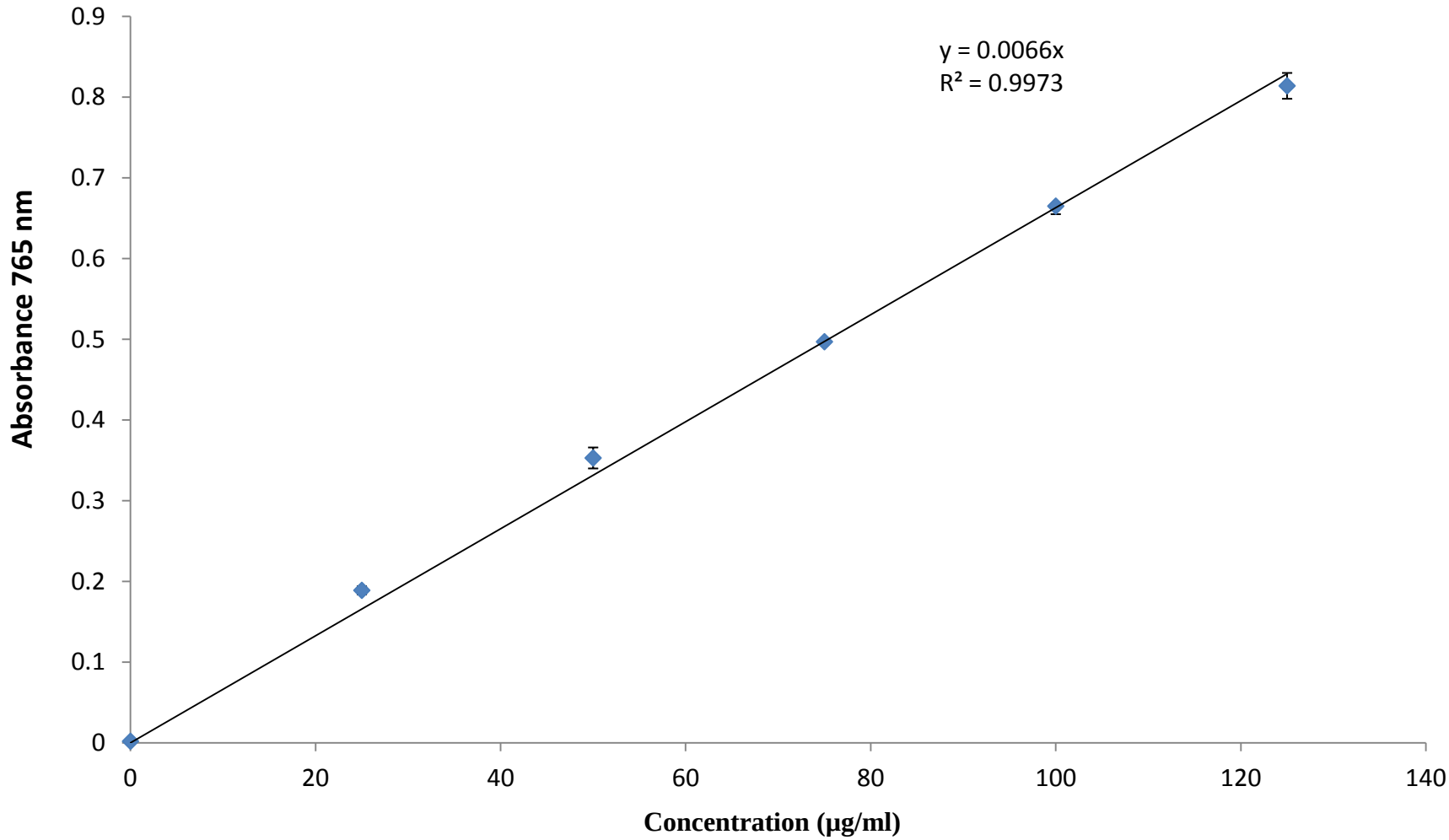


Figure 4.4.1: Graph of standard gallic acid

4.3 Determination of Antioxidant Activity of *Rhodomyrtus tomentosa* in different Crude Extracts

Determinations of antioxidant activity were carried out using 1,1-diphenyl-2-picrylhydrazyl (DPPH), ferric reducing antioxidant power (FRAP) and metal chelating ability assay. Ascorbic acid, Butylated hydroxyl toluene (BHT) and Ethylenediaminetetraacetic acid (EDTA) were standards in these assays respectively. The antioxidant activities were different between different extracts. All three assays showed that methanol extract had the most antioxidant capability followed by water extract, chloroform extract and lastly petroleum ether extract in. DPPH assay demonstrated that methanol extract had lower IC_{50} (107µg/ml) compared to IC_{50} of water extract (154µg/ml). The FRAP assay revealed methanol extract as the most antioxidant capability with absorbance of 0.162nm at concentration of 500µg/ml. In metal chelating ability assay also showed methanol extract with the highest chelating capability with 36% at concentration of 100µg/ml.

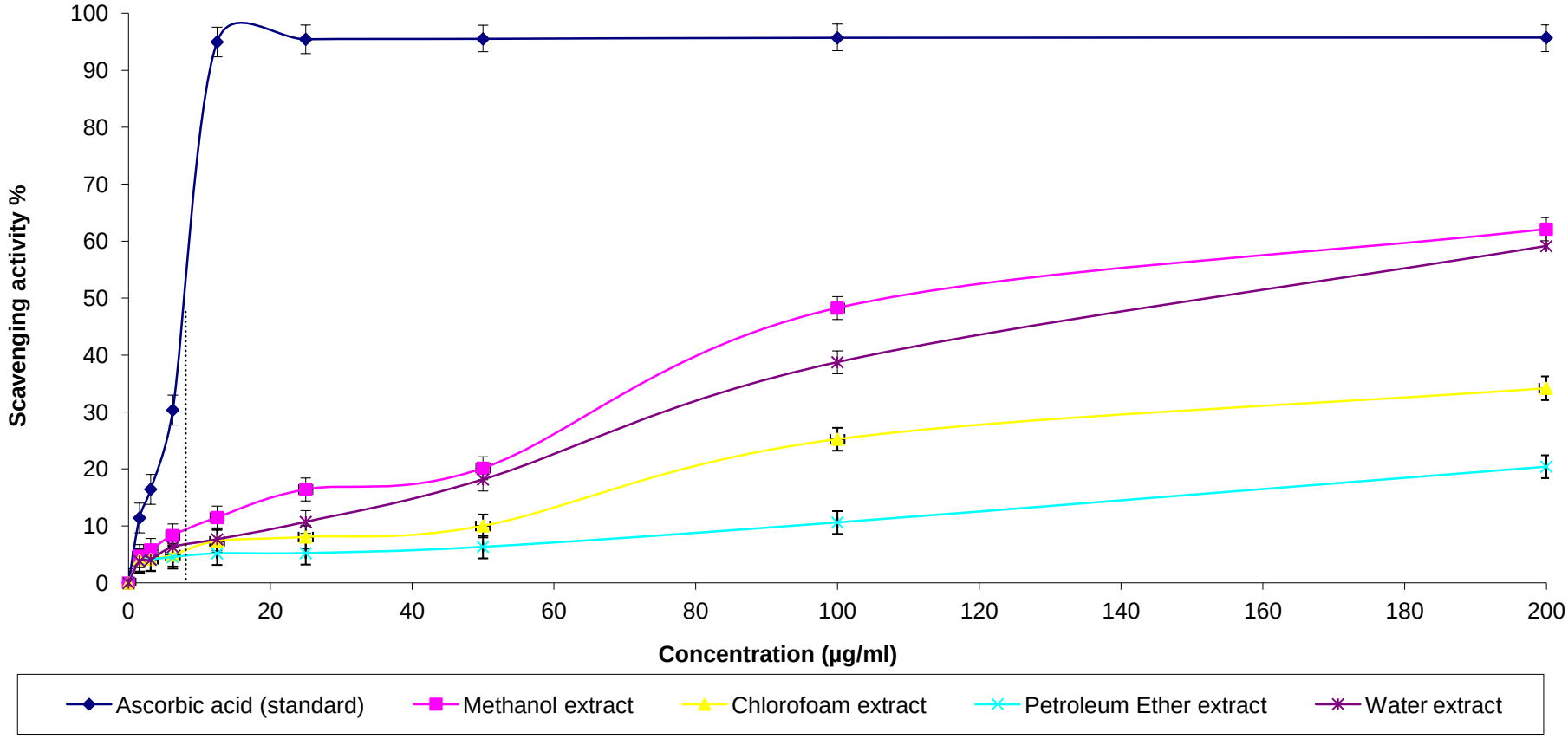


Figure 4.3.1: Free-radical scavenging activity of crude extract from fruits of *R. tomentosa* measured by DPPH assay. Data are mean ± SD (n=3)

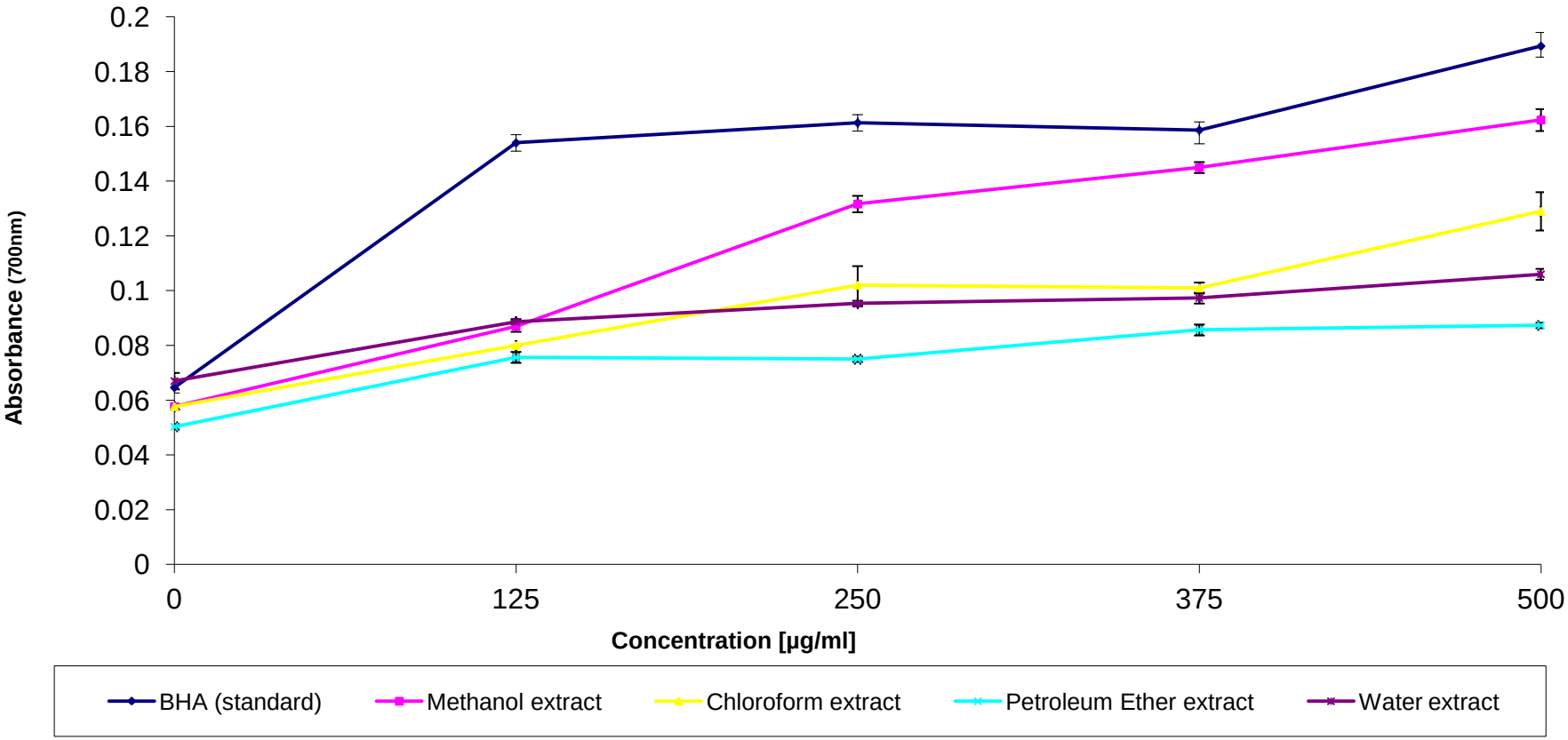


Figure 4.3.2: Ferric Reducing Antioxidant Power of crude extract from fruits of *R. tomentosa*. Data are mean ± SD (n=3)

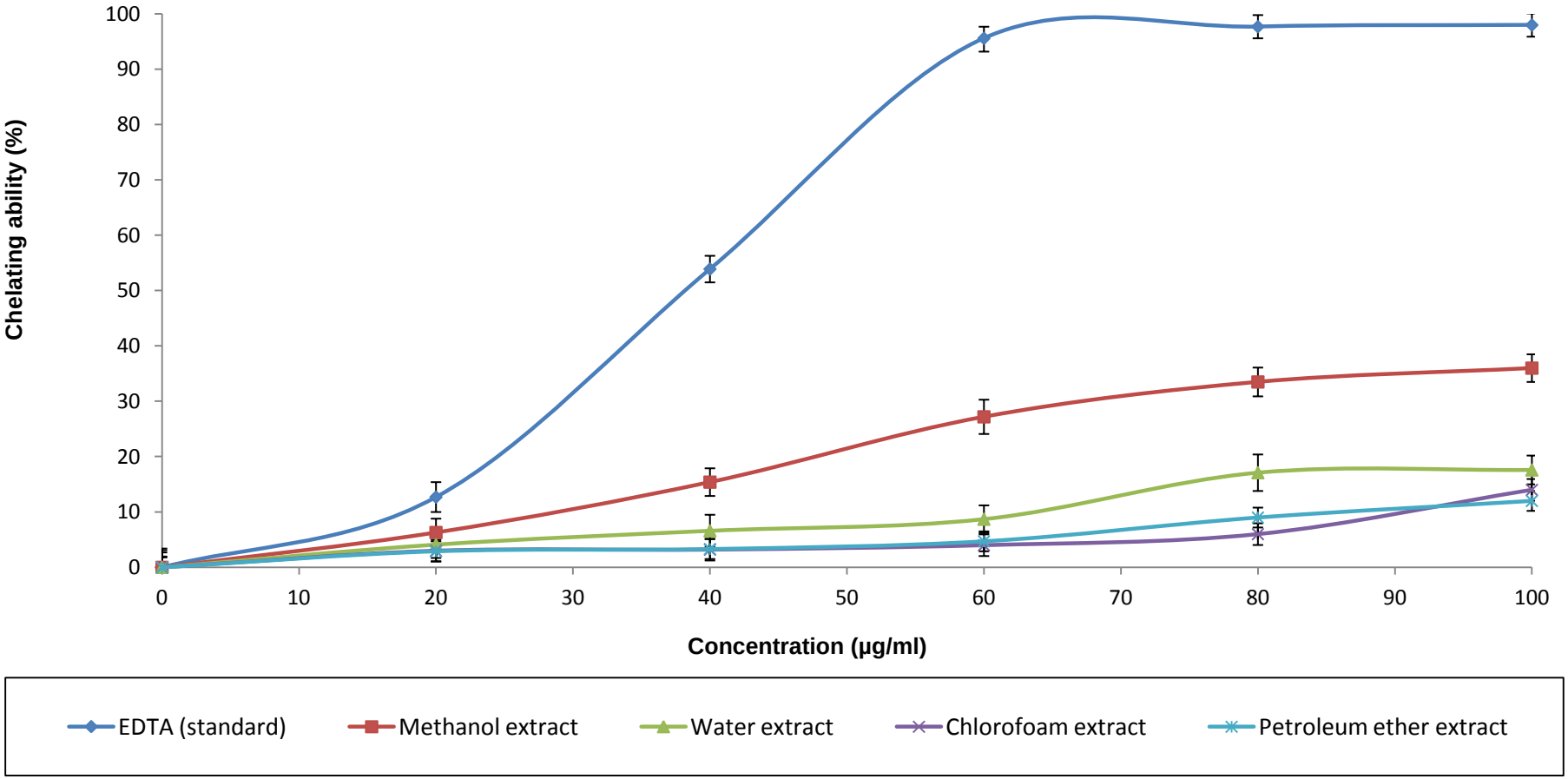


Figure 4.3.3: Metal Chelating Ability of the crude extract from the fruit of *R. tomentosa*. Data are mean ± SD (n=3)

4.5 Determination of Total Flavonoid Contents (TFC) in *Rhodomytus tomentosa* crude extract

Table 4.5.1: Total Flavonoid Contents of different *Rhodomyrtus tomentosa* extracts. Data presented are in mean ± standard error (n = 3)

Extracts	Total Flavonoid Contents (mg/ml)
Water	1.828 ± 0.018
Methanol	1.602 ± 0.003
Chloroform	1.499 ± 0.003
Petroleum Ether	0.395 ± 0.010

Total flavonoid content (TFC) of water extract (1.828 ± 0.018mg/ml) was higher than that on methanol extract (1.602 ± 0.003mg/ml). Followed by chloroform extract (1.499 ± 0.003mg/ml) and lastly petroleum ether (0.395 ± 0.010mg/ml). Quercetin was used as standard and the standard graph had y = 0.5405x and R^2 with 0.9952.

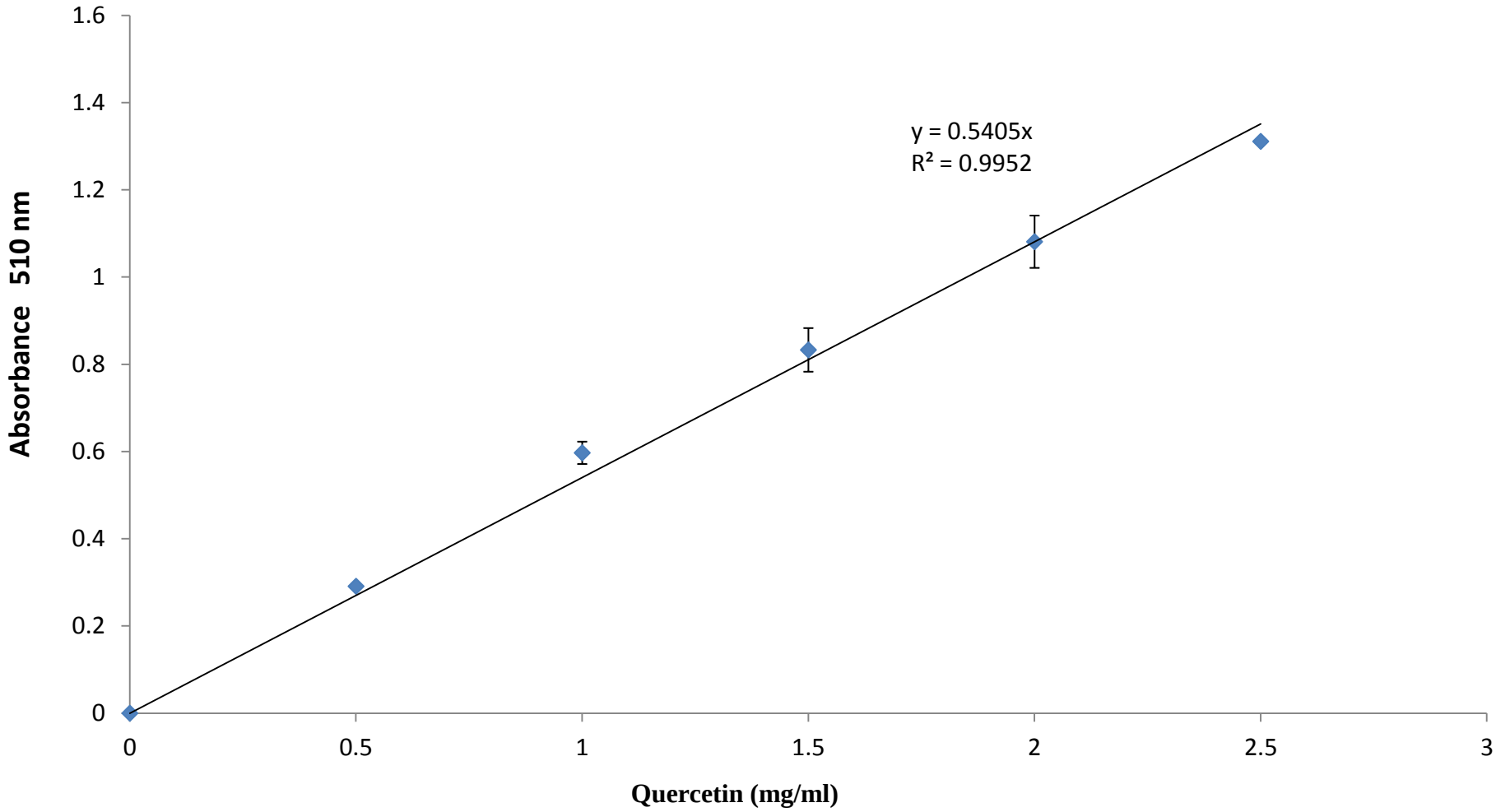


Figure 4.5.1: Graph of standard quercetin

4.6 Brine Shrimp lethality Assay

Brine Shrimp Lethality Assay (BSLA) is a general bioassay which can be used for detecting in wide range of biological activities, pharmacological activities and chemical toxicity. BSLA was used because this assay provides the preliminary assay to evaluate the toxicity of plants (Meyer *et al.*, 1982). *Artemia salina* is a simple zoological organism which has sensitivity to toxic environment. In vivo, lethality of such toxic environment can be used as a convenient method to monitor and screening bioactive compounds. *Artemia salina* brine shrimp has the advantages of being simple, rapid yet inexpensive. Furthermore it does not require any blood, tissues or large animals to be sacrificed in order to obtain toxicity effect of particular compounds. LC_{50} for crude water extract, crude methanol extract, chlorofoam extract and petroleum extract were 616.083µg/ml**,** 316.228µg/ml, 100µg/ml and 31.623µg/ml respectively

Table 4.6.1: LC_{50} of crude water extract of *Rhodomyrtus tomentosa*

Concentration Sample [µg/ml]	**Total No of Shrimp**	**Number of Dead**	**Percentage Mortality (%)**	**LC_{50} [µg/ml]**	**95 percent confidence**
1000	10	6	60	616.083	141.674 – 1215910
100	10	2	20		
10	10	1	10		

Table 4.6.2: LC_{50} of crude methanol extract of *Rhodomyrtus tomentosa*

Concentration Sample [µg/ml]	**Total No of Shrimp**	**Number of Dead**	**Percentage Mortality (%)**	**LC_{50} [µg/ml]**	**95 percent confidence**
1000	10	7	70	316.228	75.036 – 14612.65
100	10	3	30		
10	10	1	10		

Table 4.6.3: LC_{50} of crude chlorofoam extract of *Rhodomyrtus tomentosa*

Concentration Sample [µg/ml]	**Total No of Shrimp**	**Number of Dead**	**Percentage Mortality (%)**	**LC_{50} [µg/ml]**	**95 percent confidence**
1000	10	8	80	100	11.413 – 876.213
100	10	5	50		
10	10	2	20		

Table 4.6.4: LC_{50} of crude petroleum ether extract of *Rhodomyrtus tomentosa*

Concentration Sample [µg/ml]	**Total No of Shrimp**	**Number of Dead**	**Percentage Mortality (%)**	**LC_{50} [µg/ml]**	**95 percent confidence**
1000	10	10	100	31.623	4.706 – 89.698
100	10	7	70		
10	10	3	30		

4.7 Maximum Tolerated Dose (MTD) of *Rhodomyrtus tomentosa* on rabbits

Table 4.7.1: Maximum tolerated dose of water extract of *Rhodomyrtus tomentosa* based on body weight effect of male rabbit.

Rabbit	Concentration mg/kg	Duration of treatment (Week)					Weight Gain/Loss (g)
		0	1	2	3	4	
1	Control	2040	2080	2130	2230	2395	355 ± 63.522
2	50	2110	2255	2330	2375	2450	340 ± 57.909
3	100	2100	2180	2235	2300	2365	265 ± 46.027
4	500	2155	2180	2250	2315	2390	235 ± 43.261

Values are expressed as mean ± SE. No statistical difference between control and *R. tomentosa* treated group ($p<0.05$).

Table 4.7.2: Maximum tolerated dose of water extract of *Rhodomyrtus tomentosa* based on body weight effect of female rabbit

Rabbit	Concentration mg/kg	Duration of treatment (Week)					Weight Gain/Loss (g)
		0	1	2	3	4	
1	Control	2115	2170	2245	2360	2435	320 ± 59.097
2	50	2205	2255	2330	2455	2500	295 ± 56.555
3	100	2140	2235	2380	2440	2505	365 ± 67.026
4	500	2105	2125	2205	2315	2476	371 ± 68.506

Values are expressed as mean ± SE. No statistical difference between control and *R. tomentosa* treated group ($p<0.05$).

Table 4.7.3: Maximum tolerated dose of methanol extract of *Rhodomyrtus tomentosa* based on body weight effect of male rabbit

Rabbit	Concentration mg/kg	Duration of treatment (Week)					Weight Gain/Loss (g)
		0	1	2	3	4	
1	Control	2085	2120	2195	2235	2295	210 ± 37.98
2	50	2035	2090	2130	2165	-	130 ± 27.91
3	100	2010	2070	2140	2175	-	165 ± 36.76
4	500	2025	2060	2120	-	-	95 ± 27.74

Values are expressed as mean ± SE. No statistical difference between control and *R. tomentosa* treated group ($p<0.05$).

Table 4.7.4: Maximum tolerated dose of methanol extract of *Rhodomyrtus tomentosa* based on body weight effect of female rabbit.

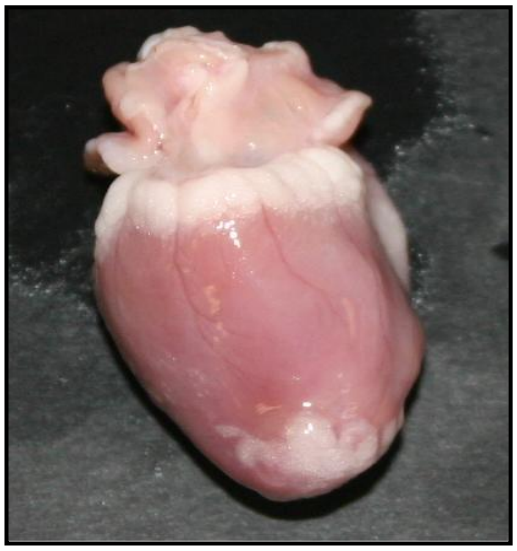
Rabbit	Concentration mg/kg	Duration of treatment (Week)					Weight Gain/Loss (g)
		0	1	2	3	4	
1	Control	2010	2075	2115	2190	2235	225 ± 40.94
2	50	2015	2070	2100	2135	-	120 ± 25.41
3	100	2040	2095	2105	-	-	65 ± 20.21
4	500	2055	2110	2145	-	-	90 ± 26.19

Values are expressed as mean ± SE. No statistical difference between control and *R. tomentosa* treated group ($p<0.05$).

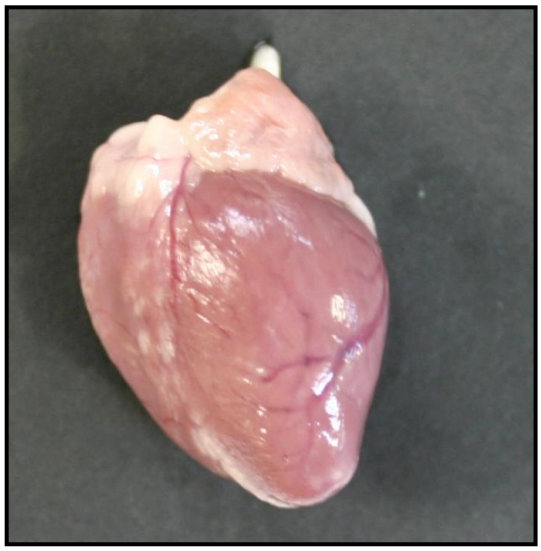
The results presented in the table 4.7.1, 4.7.2, 4.7.3 and 4.7.4 show maximum tolerated dose of water and methanol extracts of *R. tomentosa* on female and male rabbits. Toxicology was assessed on the mortality rate. Water extract of dosages of 50, 100, and 500mg/kg/day did not produce any mortality to the rabbits. Methanol extract at dosage of 50, 100 and 500mg/kg/day showed mortality at week 3 and 4. From this experiment it can be concluded that the rabbits did not have mortality even at maximum dosage of 500mg/kg/day. Therefore using multiple dose levels might not be necessary for further in vivo experiment.

4.8 Animal Organ Parameter

Heart



(A)



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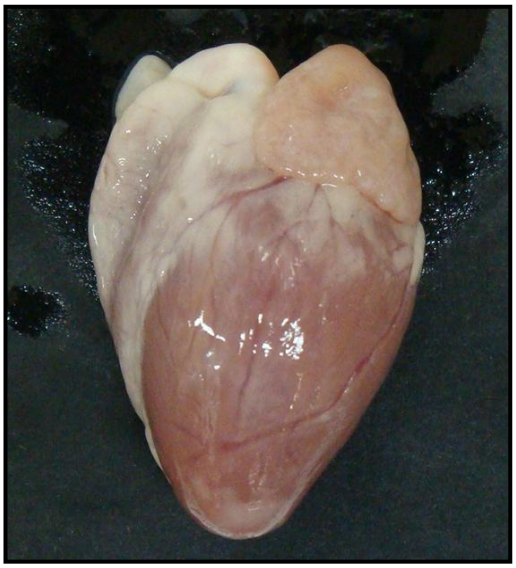
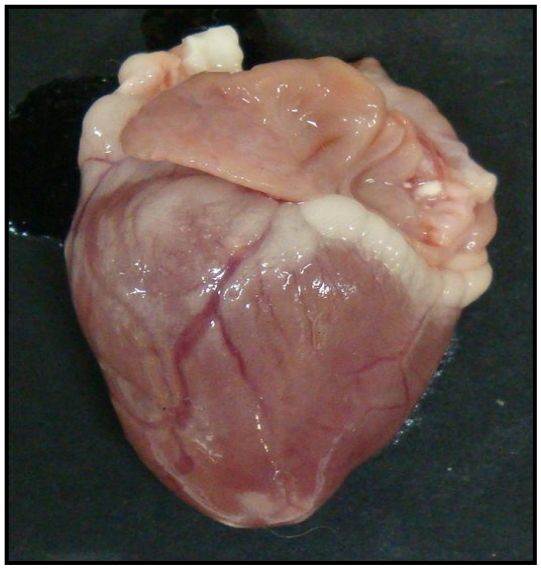


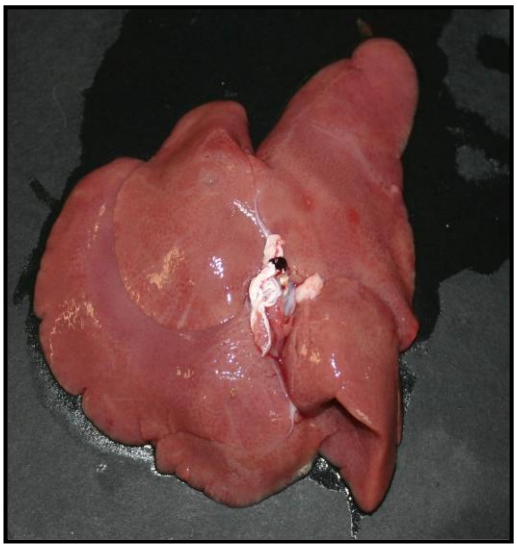
(C)



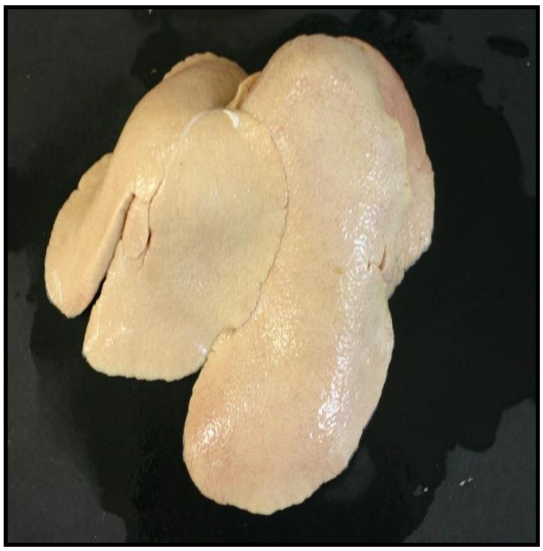
(D)

Figure 4.8.1: Dissected heart organs (A) = Normal group (B) = Cholesterol group (C) = Tomentosa group (D) = Simvastatin group

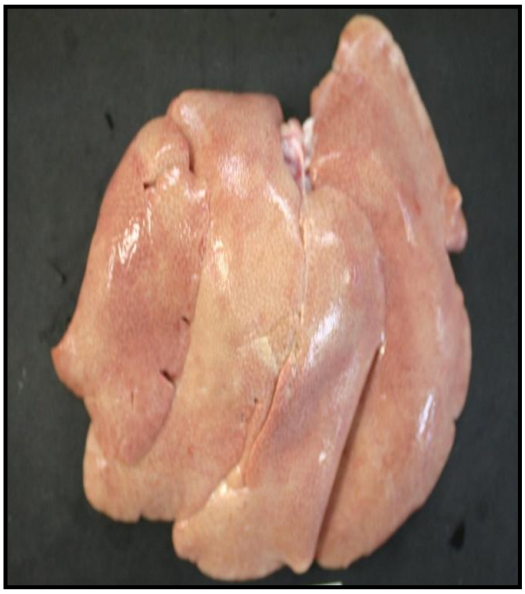
Liver



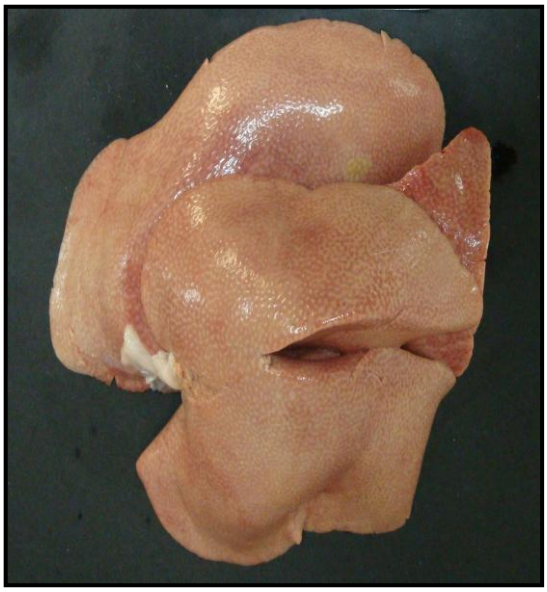
(A)



(B)



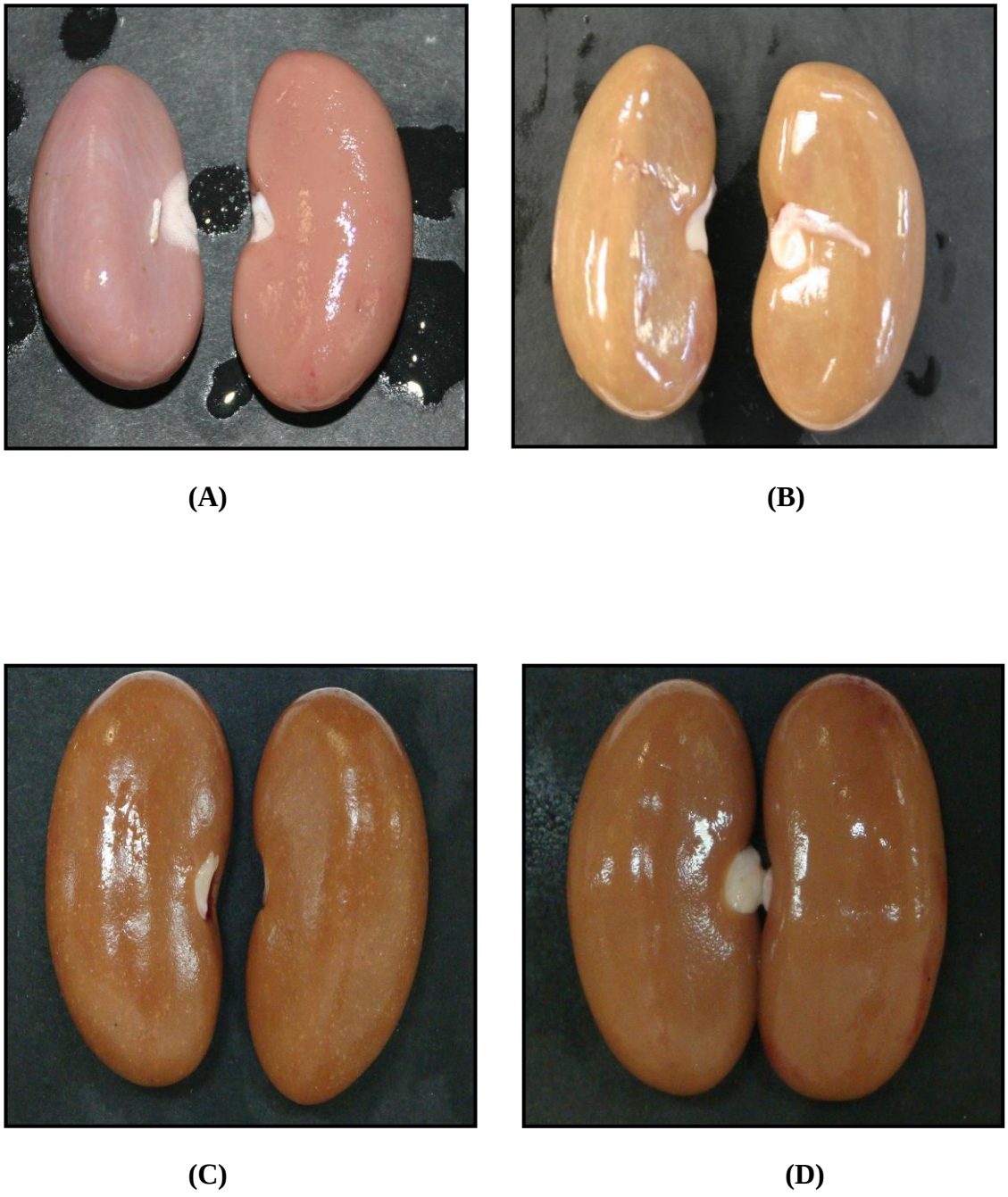
(C)



(D)

Figure 4.8.2: Dissected liver organs (A) = Normal group (B) = Cholesterol group (C) = Tomentosa group (D) = Simvastatin group

Kidney



(A) **(B)**

(C) **(D)**

Figure 4.8.3: Dissected kidney organs (A) = Normal group (B) = Cholesterol group (C) = Tomentosa group (D) = Simvastatin group

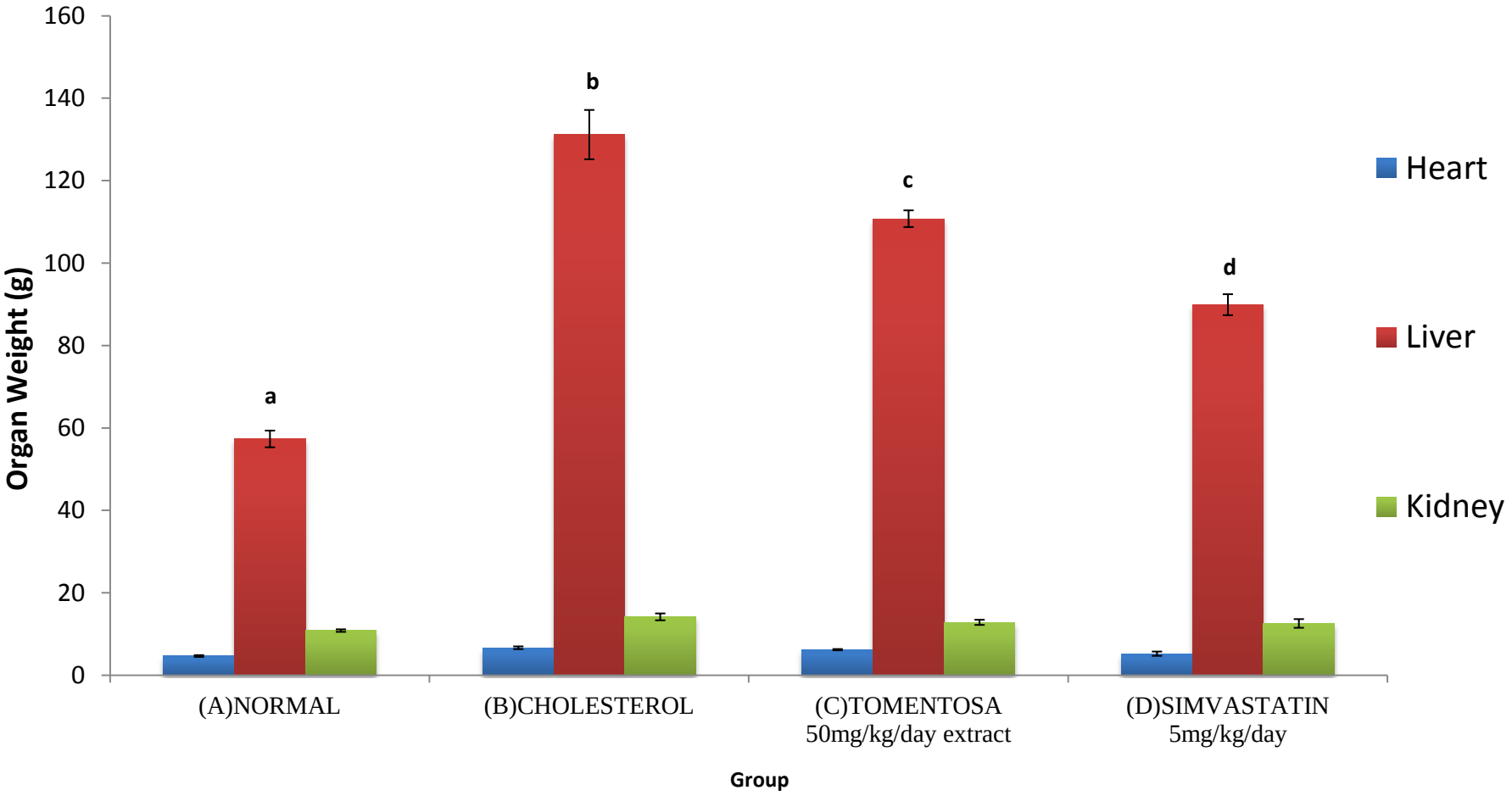


Figure 4.8.4: Heart, Liver and Kidney weight of experimental rabbits. Different alphabets (a), (b), (c) and (d) represent significantly different between groups ($p<0.05$). Data are expressed as mean ± S.E (n=6).

In figure 4.8.4 showed that no significant difference of heart and kidney weight observed among normal, cholesterol, tomentosa and simvastatin group at the end of treatment. However, liver had a significant difference between groups ($p<0.05$). Cholesterol group had the highest liver weight while the normal group had the lowest liver weight. Supplementation of 1% cholesterol diet did give effect on the weight of the liver. The liver seen in the figure 4.8.2 had a whitish feature due to accumulation of cholesterol in the hepatocytes. Rabbits supplemented with *R. tomentosa* extract 50mg/kg/day and simvastatin 5mg/kg/day had a significantly lower liver weight ($p<0.05$) than the cholesterol group.

4.9 The Effect of *Rhodomyrtus tomentosa* aqueous extract on Body Weight

During the course of this experiment, normal group was given 100 g/day normal pellet. The cholesterol, simvastatin and *R. tomentosa* groups were given 100 g/day special feed pellet with 1% cholesterol. As seen in figure 4.9.1, the average readings of week 0 were taken as baseline body weight in this study. The range of body weight for all groups was between 2.0kg to 2.595kg with the average of 2.208 ± 0.036kg. As the experiment progressed to week 5, there was only a slight significant increase ($p<0.05$) effect on body weight in cholesterol group, *R. tomentosa* group and simvastatin group compared to normal group. However, towards the end of week 10, all groups had significant different of body weight ($p<0.05$). The cholesterol group was the heaviest group with mean average of 2.873 ± 0.061 kg.

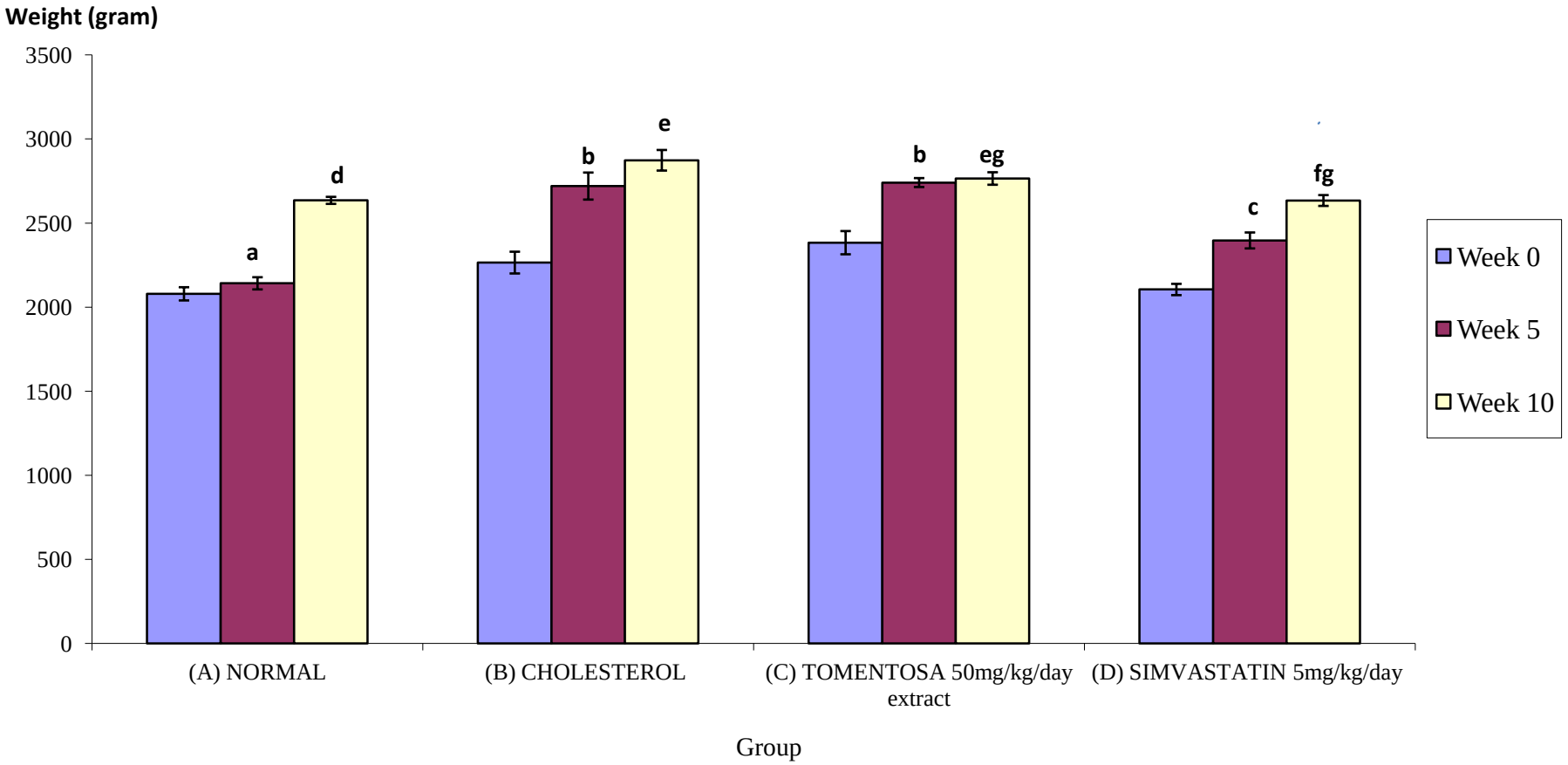


Figure 4.9.1: The effect of *R. tomentosa* water extract on body weight at different week. Different alphabets (a), (b) and (c) represent significantly different between groups at week 5 ($p<0.05$). Different alphabets (d), (e), (f) and (g) represent significantly different between groups at week 10 ($p<0.05$). Data are expressed as mean ± S.E (n=6).

The Effect of *Rhodomyrtus tomentosa* aqueous extract on Serum Triacylglycerides (TG)

The estimation of TG level at week 0 shown in the figure 4.9.2 can be used as baseline value as they are no significant difference ($p<0.05$) in each group. The average level of TG at week 0 was 0.462 ± 0.028mmol/L with range of 0.400mmol/L to 0.533mmol/L. Towards week 5, cholesterol group showed significant difference ($p<0.05$) on the level of TG at 1.3 ± 0.150mmol/L compared with the normal group, *R. tomentosa* group and simvastatin group. Later in Week 10, all groups showed insignificant level of TG ($p<0.05$) with the highest level was in *R. tomentosa* group at 0.895 ± 0.223mmol/L. There was significant decrease ($p<0.05$) of triacylglycerides in cholesterol group when comparing between week 5 and week 10.

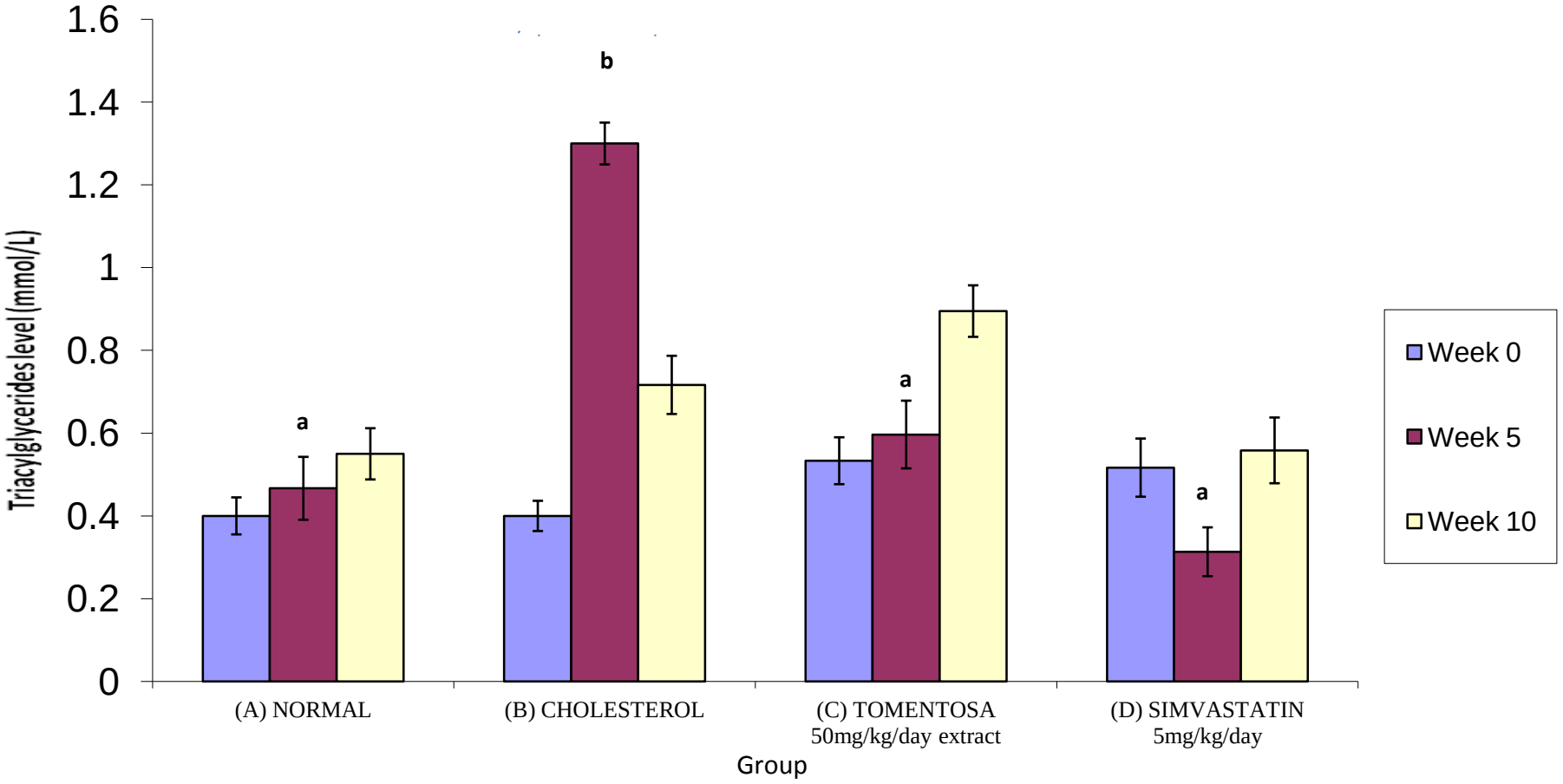


Figure 4.9.2: The effect of *R. tomentosa* water extract on Triacylglycerides (TG) at different week. Different alphabets (a) and (b) represent significantly different to its week 5 ($p<0.05$). Data are expressed as mean ± S.E (n=6).

The Effect of *Rhodomyrtus tomentosa* Aqueous Extract on Total Cholesterol (TC).

The average of serum total cholesterol level (TC) for all groups was taken at week 0 as baseline for estimation in this study. The average level at week 0 was found to be 1.204 ± 0.074mmol/L with range between 0.950mmol/L to 1.383mmol/L. As shown in figure 4.9.3, the TC level at week 0 showed no significant different ($p<0.05$) in every control group (normal and cholesterol) and treatment group (*R. tomentosa* extract and simvastatin). At week 5, all groups except normal group exhibited significant high TC levels ($p<0.05$). Cholesterol group showed the highest level of TC among all groups at 24.833 ± 0.138mol/L. Followed by simvastatin group 15.967 ± 3.402mmol/L and *R. tomentosa* group 12.633 ± 1.682mmol/L. At week 10, *R. tomentosa* group (17.05 ± 0.419mmol/L) and simvastatin group (16.067 ± 0.651mmol/L) showed significant lower of TC level ($p<0.05$) compared to cholesterol group (25.983. ± 0.575mmol/L).

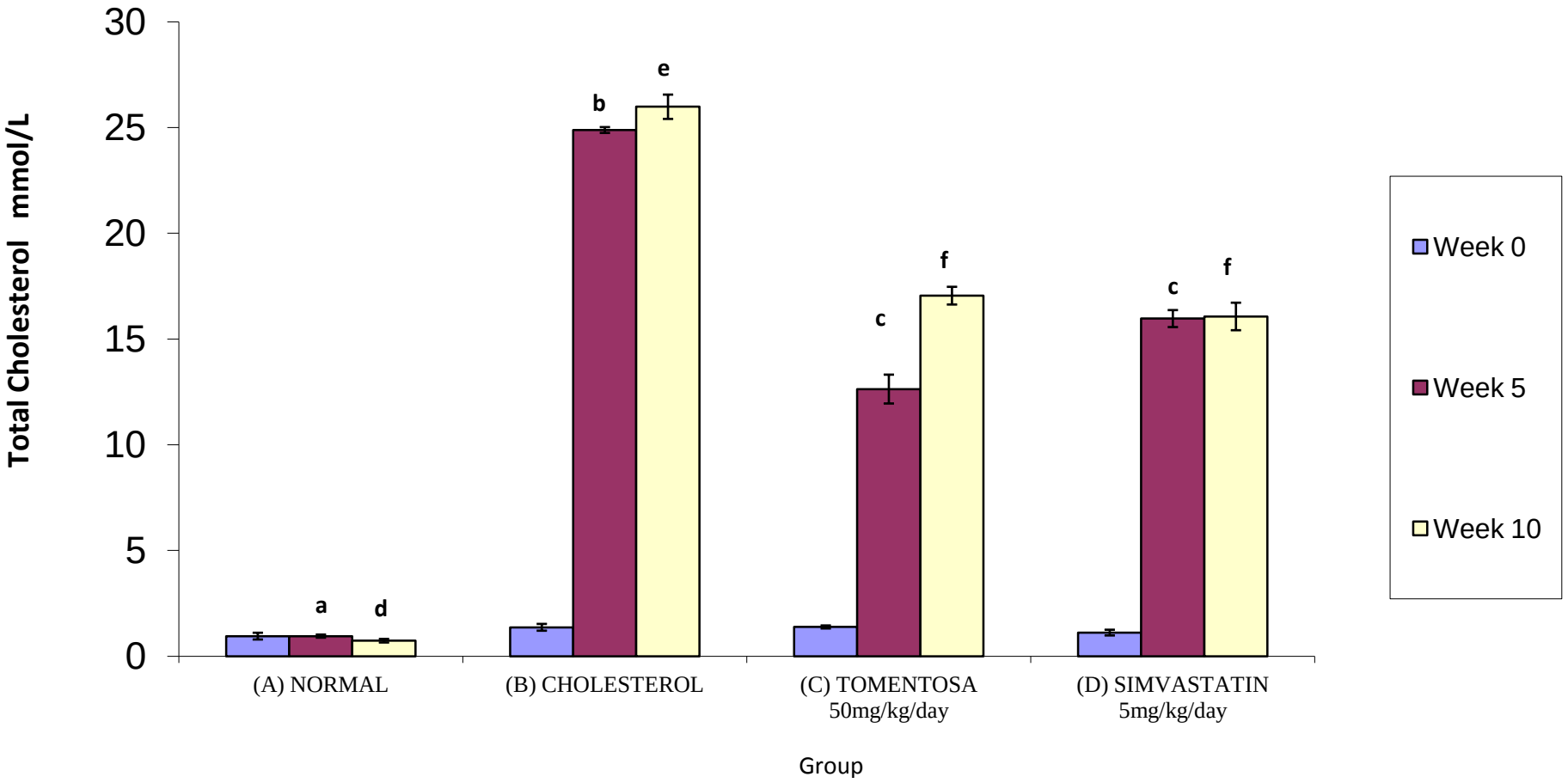


Figure 4.9.3: The effect of *R. tomentosa* water extract on Total Cholesterol (TC) at different week. Different alphabets (a), (b) and (c) represent significantly different between groups at week 5 ($p<0.05$). Different alphabets (d), (e) and (f) represent significant different between groups at week 10 ($p<0.05$). Data are expressed as mean ± S.E (n=6).

The effect of *Rhodomyrtus tomentosa* Aqueous Extract on Serum Low Density Lipoprotein (LDL).

In figure 4.9.4, the estimation of LDL level at week 0 can be used as baseline value as all level in each group showing no significant different ($p<0.05$). The average level of LDL was 0.36 ± 0.049mmol/L with range of 0.232 mmol/L to 0.448 mmol/L. At week 5, all groups except normal group showed significant increase ($p<0.05$) level of LDL. Cholesterol group had the significantly highest level ($p<0.05$) of LDL 23.295 ± 0.136mmol/L, followed by *R. tomentosa* group 11.647 ± 1.732mmol/L and simvastatin group 15.305 ± 3.159mmol/L. The LDL level at week 10 showed significant lower ($p<0.05$) in *R. tomentosa* group and simvastatin group compared with cholesterol group but slightly higher when compared to week 5 for each groups. The LDL level for *R. tomentosa* and simvastatin at week 10 was at 17.572 ± 0.341mmol/L and 15.293 ± 0.675mmol/L.

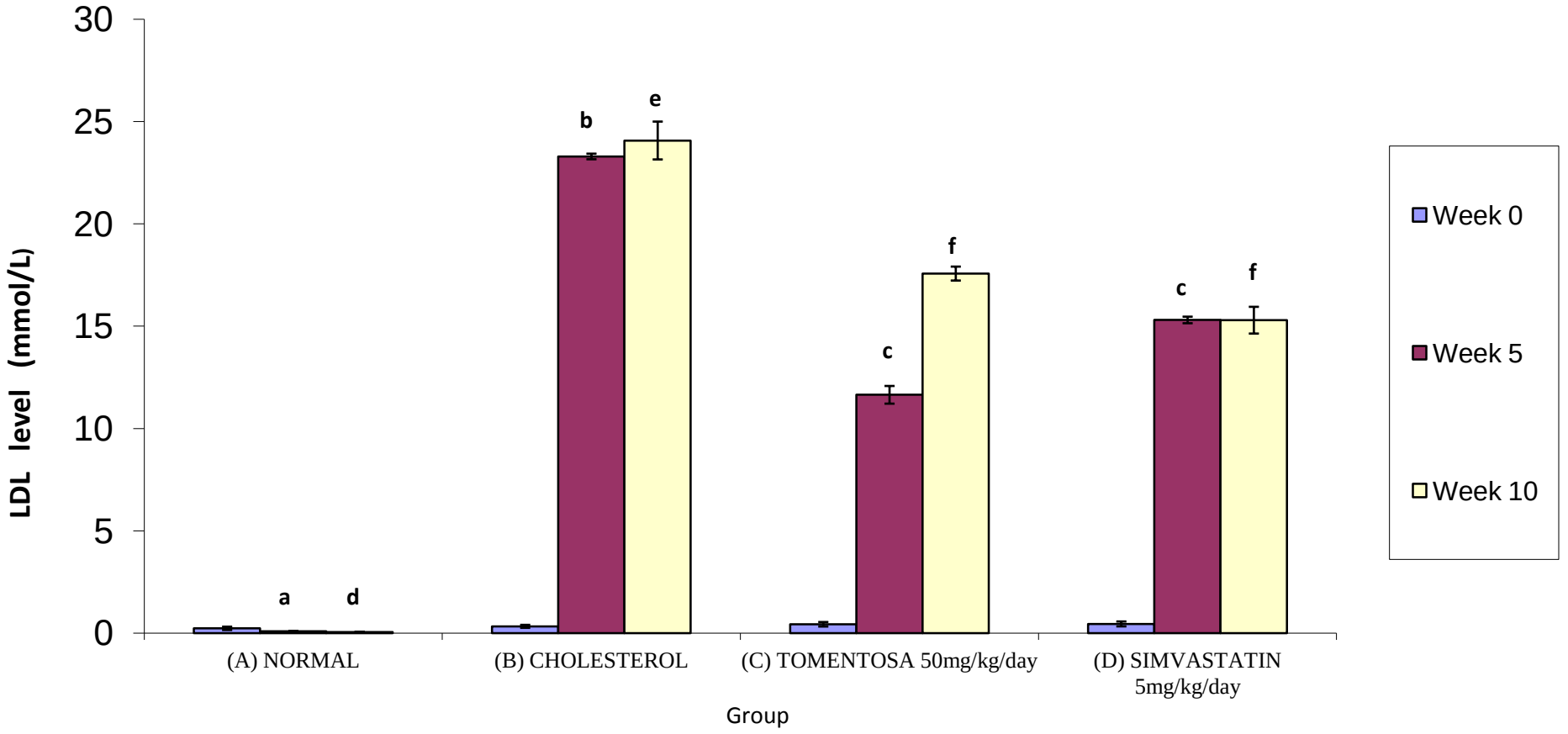


Figure 4.9.4: The effect of *R. tomentosa* water extract on Low Density Lipoprotein (LDL) at different week. Different alphabets (a), (b), and (c) represent significantly different between groups at week 5 ($p<0.05$). Different alphabets (d), (e) and (f) represent significantly different between groups at week 10 ($p<0.05$). Data are expressed as mean ± S.E (n=6).

The effect of *Rhodomyrtus tomentosa* Aqueous Extract on Serum High Density Lipoprotein (HDL).

In figure 4.9.5, the estimation of HDL level at week 0 may be used as baseline as all level in each group showed no significant different ($p<0.05$). The average of HDL level was 0.591 ± 0.024mmol/L in range of 0.527mmol/L to 0.670mmol/L. At week 5, the data showed no significant difference ($p<0.05$) in HDL level in all groups even though the *R. tomentosa* group and simvastatin group had slightly increased HDL level with 0.628 ± 0.039mmol/L and 0.612 ± 0.062mmol/L respectively. After week 10, the level of HDL in cholesterol group was decrease significantly ($p<0.05$) with value of 0.423 ± 0.038mmol/L, meanwhile *R. tomentosa* group had HDL level of 0.520 ± 0.047mmol/l which is higher than the cholesterol group. The simvastatin group had HDL level of 0.4967 ± 0.053mmol/L which also significantly higher than cholesterol group. Overall the average of HDL level in all groups at week 10 was 0.493 ± 0.030mmol/L.

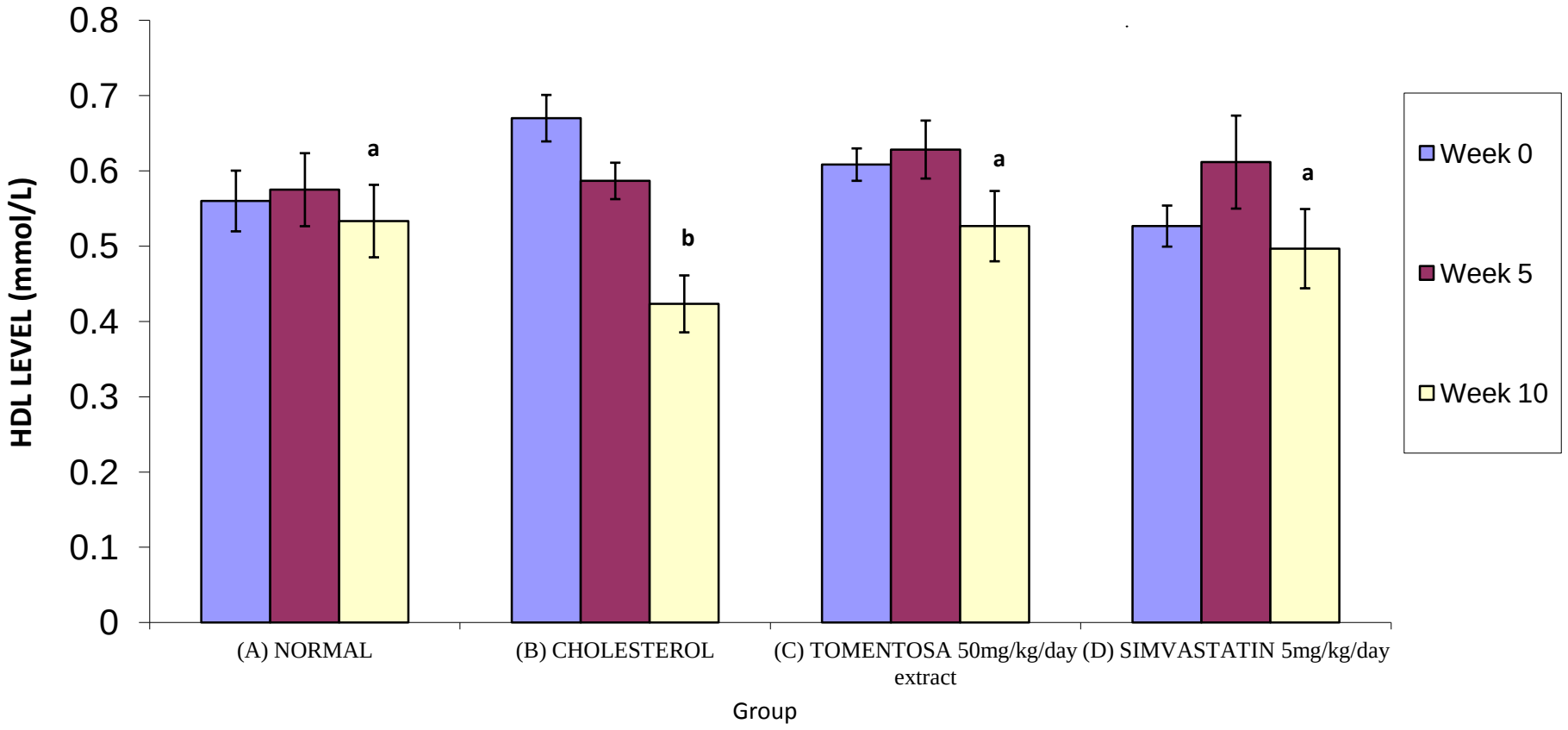


Figure 4.9.5: The effect of *R. tomentosa* water extract on High Density Lipoprotein (HDL) at different week. Different alphabets (a) and (b) represent significantly different to its week 10 ($p<0.05$) Data are expressed as mean ± S.E (n=6).

4.10 The Effect of *R. tomentosa* Aqueous Extract on Serum Lipid Peroxidation Index, TBARs-Malondialdehyde (MDA).

Studies have proven that lipid oxidation by products are MDA (Uchida, 2006) can bind and modify to various macromolecules such as proteins and lipoproteins (Yamada et al, 2001). Modifications of proteins or lipoproteins have many harmful effects in a number of diseases including atherosclerosis. This binding and modification of proteins and lipoproteins usually leads to onset of atherosclerosis (Fu *et al.,* 1998). In atheroslcrerotic lesions (Palinski *et al.*, 1989; Yla-Herttuala *et al*., 1989) and circulations (Holvoet *et al.*, 1995; Palinski *et al*., 1996) of blood, there have been detections of modified proteins and lipoproteins. The current experiment is important because MDA is one of the most important biomarker produced during lipid peroxidation. Lipid peroxidation occurs when cells are exposed to reactive oxygen species causing cell walls to rupture and membrane lipids to degrade to the end-product that is MDA.

As shown in figure 4.10.1, the levels of malondialdehyde (MDA) consist of different values among different groups. The level of MDA at week 0 cannot be considered as basal line of experiment because each group has different multiple variables. This condition occurs maybe due to any internal or external factor during assessing the MDA level in lab or maybe the subjects condition itself was not stable as the rabbits were quite young.

The average level of MDA at week 0 was 0.452 ± 0.001nmol/ml. In week 5, cholesterol group showed significant different ($p<0.05$) in MDA level compared to other groups. The level at week 5 was at 0.072 ± 0.003nmol/ml. Later in week 10, cholesterol group still showed significantly higher ($p<0.05$) in MDA level compared with other group. The level of MDA in *R. tomentosa* group and simvastatin group was at 0.043 ± 0.001nmol/ml and 0.046 ± 0.001nmol/ml. Generally, all groups showing different rate in MDA level with the exception of simvastatin group with

slightest increase. *R. tomentosa* group show the lowest level followed by simvastatin group, normal group (0.048 ± 0.003nmol/ml) and lastly cholesterol group (0.068 ± 0.001nmol/ml). The supplementation of 50mg/kg/day of *R. tomentosa* resulted in significantly lower ($p<0.05$) of MDA respectively compared to cholesterol group.

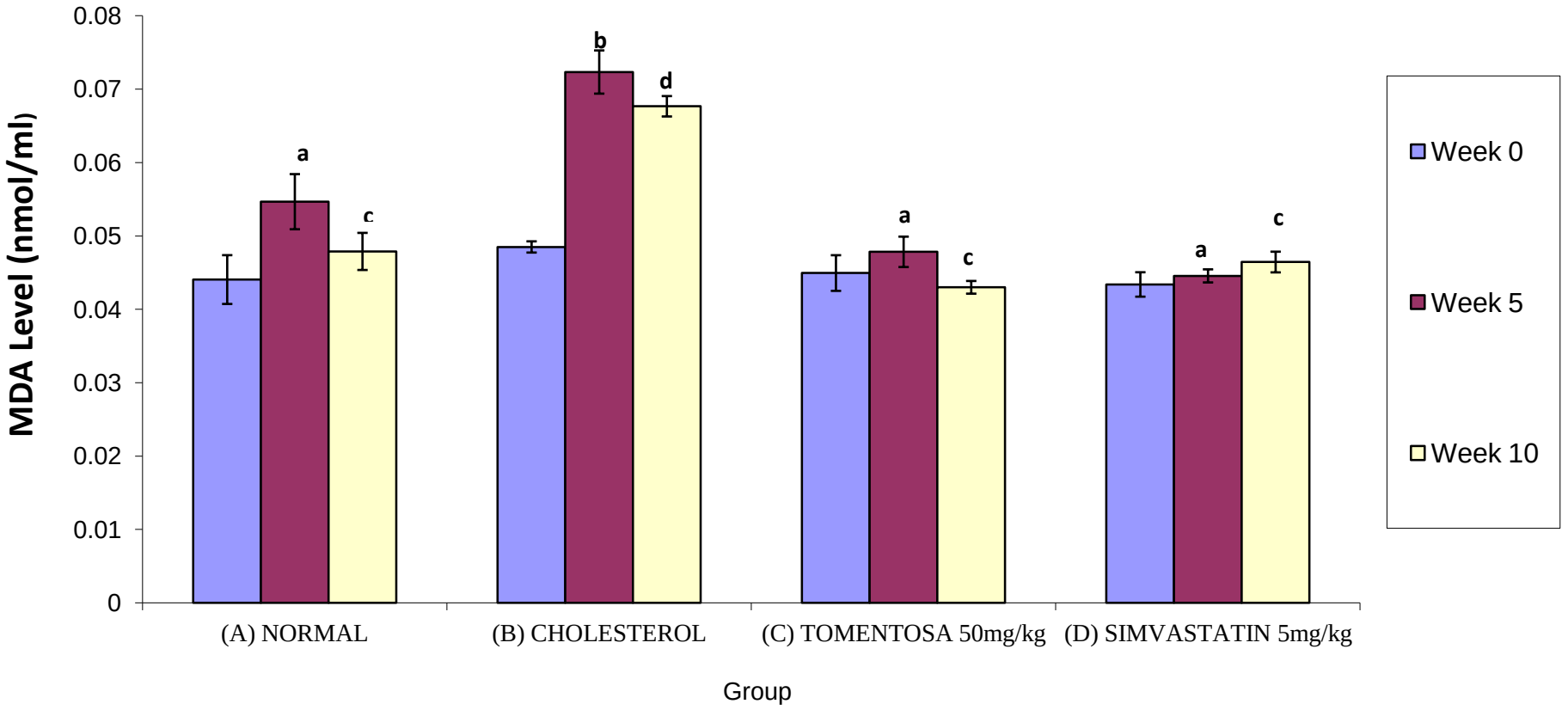


Figure 4.10.1: The effect of *R. tomentosa* water extract on lipid peroxidation index, indicated by malondialdehyde (MDA) at different time. Alphabet (a) and (b) represent significant difference at week 5 ($p<0.05$), (c) and (d) represent significant difference at week 10 ($p<0.05$). # significant different with in group. Each point represents a mean $\pm$ S.E (n=6)