4: RESULTS:

4.1: Yield of *Curcuma caesia* Roxb. rhizome extracts:

Dried rhizomes of *Curcuma caesia* Roxb. were finely grounded and extracted with different solvents starting from the least polar solvent to more polar ones such as petroleum ether, ethyl acetate, ethanol, methanol and aqueous. The extracts were filtered and concentrated under water bath and percentage yields of each extract obtained are given below in the table no.4.

Table: 4: Yield of the extracts:

Types of extracts	**Weight of crude extracts**	**Yield of the Extracts**	**Percentage yield**
Petroleum Ether Extract	100g	0.58g	0.58%
Ethyl Acetate Extract	99.42g	2.454g	2.468%
Ethanol Extract	96.96g	2.042g	2.106%
Methanol Extract	94.91g	0.947g	0.997%
Aqueous Extract	93.96g	1.452g	1.545%

4.2: Preliminary Phytochemical Screening:

Preliminary phytochemical screening of various extracts of the rhizome of *C. caesia* Roxb. reveals the presence of alkaloids, carbohydrates, flavanoids, phenolics, reducing sugars, terpenes, steroids, starch, saponins and tannins in different kinds of extracts as indicated in table no 5.

Table No: 5: Preliminary Phytochemical Screening:

Test	Methods	EaECC	EECC	MECC	AECC
1. Test for alkaloids	Dragendroff's test	-	-	+	-
	Mayer's test:	+	++	+	+
	Hager's test:	-	-	+	+
	Wagner's test:	-	+	-	-
2.Test for carbohydrates:	Molisch's test	++	+	+	-
3.Test for steroids	Libermann-Burchard Reaction:	++	+++	+	+
4.Test for Flavanoids	Aqueous NaOH test	-	-	-	+
	Concentrated H_2SO_4 test	+	+	+	+
	Schinoda's test	-	-	+	-
5.Detection of Phenolic compounds	Ferric chloride test:		++	-	+
	Lead acetate test	+	+	+	+
6.Test for Reducing Sugars	Fehling's Test for free reducing sugar	+	+	+	+
	Fehlings test for combined reducing	+	++	+	+

	sugar				
7.Test for Tannins		-	+	+	+
8.Test for Soluble Starch		-	+	-	-
9.Test for terpenoids		++	+	+	+
10. Test for Saponins		-	-	-	+

4.3: Determination of total phenol contents in the plant extracts:

The total phenol contents in the examined plant extracts using the Folin Ciocalteu's reagent is expressed in terms of Gallic acid equivalent (the standard curve: $y = 0.0178x + 0.148$; $R^2 = 0.9831$) (fig:16). Total phenolic contents in the examined extracts ranged: MECC= 52.11mg GAE/100g d.wt, EECC=68.64 mg GAE/100g d.wt, EaECC=38 mg GAE/100g d.wt, AECC= 4.82 mg GAE/100g d.wt of the extract. The highest concentration of phenols was measured in ethanolic extract followed by methanolic extract, ethyl acetate and aqueous extracts (table no.7). Absorbance of each extract to find the total phenol content and calculated amount of phenolics as well as standard curve of Gallic acid to find out the total phenol content is given below in the table no.6, table no. 7 and figure 16.

Table No: 6: Absorbance of each extracts to find the total Phenol content:

Sl.no.	EaECC	EECC	MECC	AECC
1.	0.793	1.321	1.032	0.227
2.	0.795	1.318	1.037	0.231
3.	0.795	1.307	1.037	0.232
Mean±S.D	0.794±0.0009	1.315±0.0060	1.034±0.0023	0.23±0.0021

Table: 7: Total phenolics in the studied extracts:

GAE/g	EECC	MECC	EaECC	AECC
	68.64mg/g	52.11mg/g	38mg/g	4.82mg/g

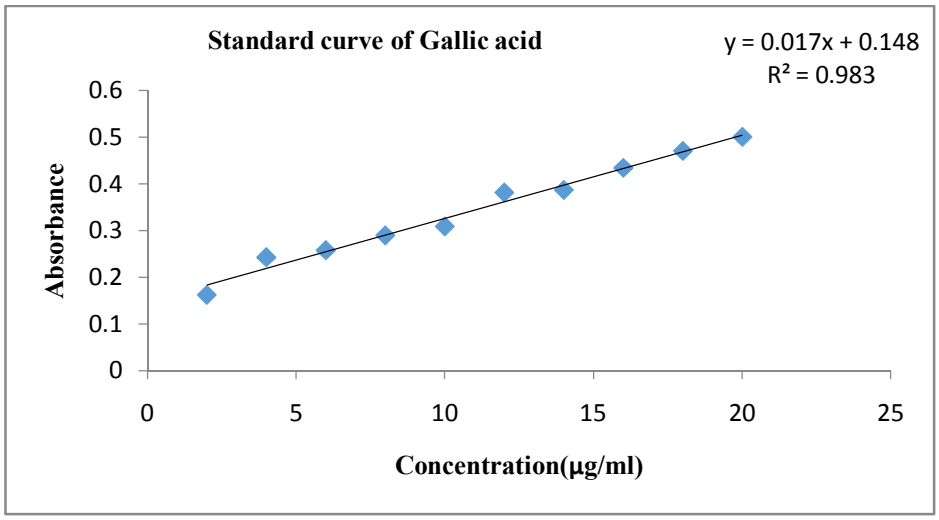


Fig: 16: Standard curve for Gallic acid to find out the total Phenol content.

4.4: Reducing power Assay:

The reducing power of *Curcuma caesia* Roxb. rhizome extracts was dose dependent and is presented in table no. 8. Themaximum absorbance of ethanolic extracts at 1000µg/mL is more or near to ascorbic acid at 200µg/mL as given in table no. 8; fig:17. Reducing power method indirectly evaluates the antioxidant activity and increase in the absorbance indicates an increase in reductive ability of the plant extracts Olayinka *et al.,* 2010.

Table: 8: Reducing power Assay:

Conc (µg/ml)	Ascorbic acid	EECC	MECC	EaECC	AECC
1000		2.480± 0.010	1.639± 0.029	0.899± 0.053	0.348± 0.023
800		2.277± 0.068	1.368± 0.029	0.468± 0.028	0.275± 0.015
500		1.511± 0.041	0.788± 0.005	0.333± 0.022	0.180± 0.009
200	2.425±0.03	0.775± 0.002	0.372± 0.001	0.159± 0.013	0.074± 0.002

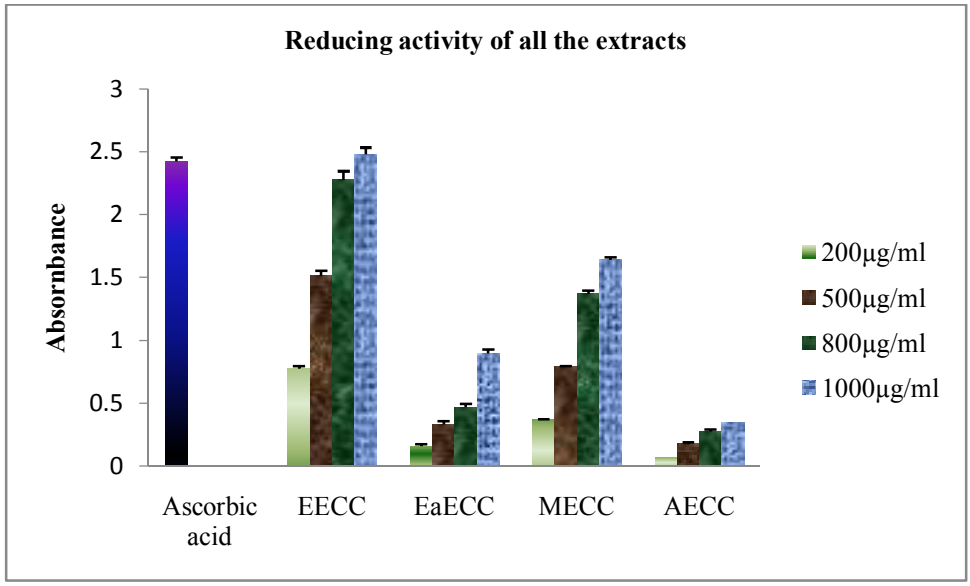


Fig: 17: Reducing power assay of *C.Caesia* Roxb extracts.

4.5: In Vitro Antioxidant Studies:

4.5.1: DPPH Radical Scavenging Assay:

The free radical scavenging activity of the rhizome extracts of *Curcuma caesia* Roxb.was measured as decolorizing activity following the trapping of the unpaired electrons of DPPH as shown in (fig:18) (table no.9). The fractions showed a varied free radical scavenging activity. The ethanol fraction was found to be the most active free radical scavenger exhibited (86.914% decrease at a concentration of 800 µg/ml) compared to ascorbic acid (94.717%) (200 µg/ml). Likewise the crude methanolic,

ethyl acetate and aqueous extract showed scavenging activity with a percent decrease of 83.104%, 70.44% and 69.19% at their highest concentration of 800 µg/ml. The IC50 value ranges in the order of 418µg/ml (EECC)>441.90 µg/ml (MECC)>561 µg/ml (EaECC)> 591µg/ml (AECC), the lowest being the highest antioxidant activity.

Table: 9: DPPH radical scavenging activity:

Conc(µg/ml)	EaECC	% Inhibition	EECC	% Inhibition	MECC	% Inhibition	AECC	% Inhibition
25	0.898± 0.004	-15.275	0.860± 0.006	-3.241	1.194± 0.006	-2.41	1.049± 0.022	53.212
50	0.855± 0.004	-9.756	0.781± 0.007	6.242	1.124± 0.006	3.602	1.194± 0.014	55.565
100	0.785± 0.007	-0.77	0.717± 0.011	13.925	1.055± 0.008	9.519	1.315± 0.469	57.918
200	0.692± 0.008	11.168	0.576± 0.030	30.852	0.824± 0.011	29.331	0.940± 0.020	60.180
400	0.510± 0.011	34.531	0.344± 0.016	58.703	0.501± 0.010	57.032	0.700± 0.014	62.624
800	0.240± 0.011	69.191	0.109± 0.009	86.914	0.197± 0.003	83.104	0.349± 0.022	64.298

Absorbance of Ascorbic acid at 200 µg/mlis 0.044 and its percentage of inhibition against DPPH is 94.717%.

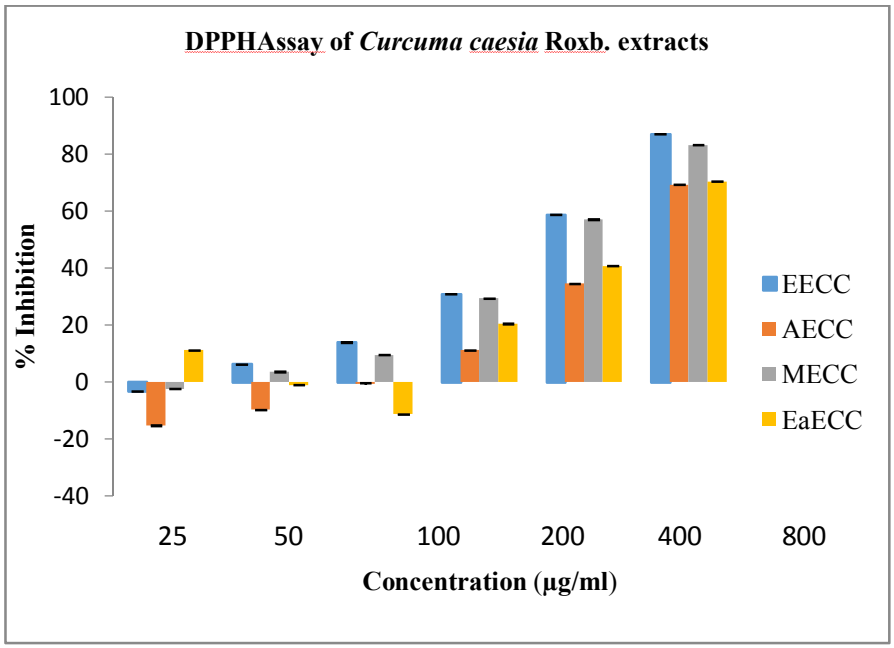


Fig: 18: DPPH Assay of *Curcuma caesia* Roxb. rhizome extracts.

4.5.2: $ABTS^+$ Radical Scavenging Activity:

The inhibitory effect of all extracts against $ABTS^+$ was compared with standard compound Gallic acid (table no. 10 and 11). The highest inhibitory effect against $ABTS^+$ was found to be exhibited by EaECC against $ABTS^+$ with the percentage inhibition of 41.15% at its lowest concentration (5µg/ml) and 77.93% at its highest concentration (100 µg/ml) which lies in between 70.57% and 92.78% of Gallic acid standard at the concentrations of 60µg/ml and 80µg/ml. The IC50 value of EaECC is 14.44 µg/ml as compared to that of Gallic acid (4.37µg/ml). The highest inhibitory effect of other extracts EECC, MECC and AECC was shown at their highest concentration (100 µg/ml) with the percentage inhibition of 63.76 %, 64.78% and 54.73% with the IC50 value of 43.63µg/ml, 51.994 µg/ml and 76.992 µg/ml respectively (table no 11). IC 50 values were calculated by plotting linear graph (taking linear equation of the graph) between the percentage inhibition and concentrations of all the extracts and standard.

Table: 10: ABTS^{+} radical scavenging activity of standard and EaECC extract of *Curcuma caesia* Roxb:

ConcGallic Acid			EaECC	
(µg/ml)	Absorbance	% Inhibition	Absorbance	%Inhibition
5	0.980±0.002	53.79	1.248±0.006	41.15
10	0.872±0.003	58.88	0.985±0.005	53.6
20	0.792±0.002	62.65	0.984±0.002	53.6
40	0.774±0.002	63.5	0.903±0.002	57.42
60	0.624±0.002	70.57	0.726±0.004	65.77
80	0.153±0.006	92.78	0.684±0.002	67.75
100	0.139±0.003	93.44	0.468±0.119	77.93

Table: 11:ABTS^{+} radical scavenging activity of EECC, MECC and AECC of *Curcuma caesia* Roxb:

ConcEECC			MECC		AECC	
(µg/ml)	Absorbance	% Inhibition	Absorbance	% Inhibition	Absorbance	% Inhibition
5	1.452±0.003	31.54	1.472±0.004	30.59	1.880±0.045	11.36
10	1.253±0.004	40.92	1.698±0.003	30.69	1.830±0.002	13.71
20	1.152±0.005	45.68	1.253±0.004	40.92	1.698±0.003	19.94
40	1.091±0.001	48.56	1.115±0.039	47.43	1.698±0.003	30.69
60	0.945±0.003	55.44	0.985±0.005	53.6	1.031±0.146	51.39
80	0.753±0.004	64.49	0.984±0.002	63.5	0.984±0.02	53.6
100	0.705±0.004	63.76	0.747±0.004	64.78	0.960±0.003	54.73

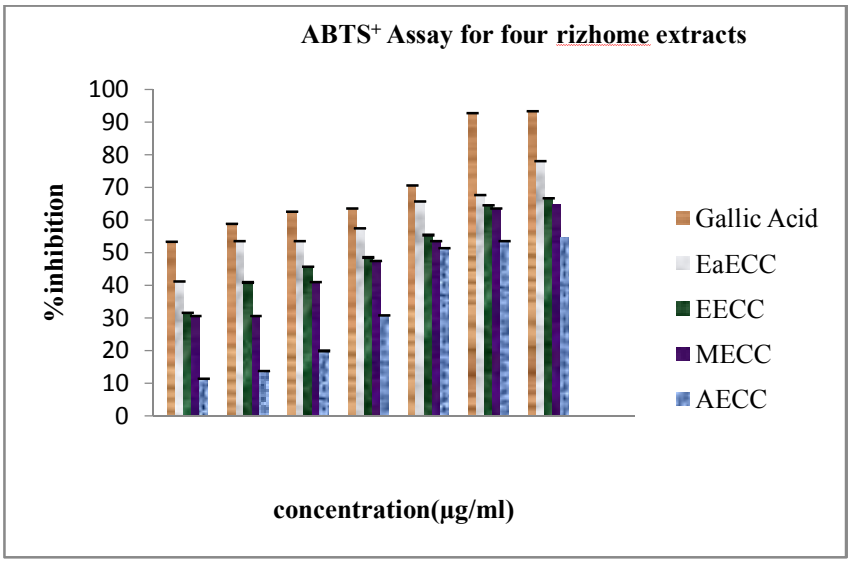


Fig: 19:ABTS$^+$ Assay for four rhizome extracts of *Curcuma caesia* Roxb.

The percentage inhibition of the Gallic acid standard was highest as compare to the extracts tested however amongst all other extracts ethyl acetate extract showed highest inhibition against ABTS$^+$ free radicals. All the extracts showed dose dependent inhibition against ABTS$^+$ free radical in the order of EaECC> EECC> MECC> AECC (figure:19; table 10 and 11).

4.5.3: Superoxide Anion Radical Scavenging Assay:

Table 12 and 13 and fig: 20; depicts the superoxide anion scavenging ability of different extracts of *C.caesia* Roxb. and standard Gallic acid. It was found that among the different extracts, ethyl acetate (EaECC) fraction of rhizomes was the most effective with an inhibitory effect of 77.88% at the concentration of 20µg/ml, whereas EECC, MECC and AECC exerted the inhibition percentage of 75.26%, 71.83% and 60.7% respectively at the same concentration. EaECC exhibits 89.77% at the concentration of 200µg/ml while EECC, MECC and AECC exerted the percent inhibition of 88.36%, 84.03% against O_2^- anion radical respectively. Gallic acid standard showed maximum O_2^- anion radical scavenging activity with the percentage inhibition of

94.05% at its highest concentration of 200µg/ml. Figure 20 shows the efficacy of different extracts of *C.caesia* Roxb to scavenge the superoxide anion radicals generated in PMS-NADH-NBT system. It was concluded that *C.caesia* Roxb extracts have shown dose dependent response.

Table: 12:Superoxide anion scavenging ability of Standard and Ethyl acetate extract of *Curcuma caesia* Roxb:

Conc(µg/ml)	Gallic acid		EaECC	
	Mean± S.D	% Inhibition	Mean ± S.D	% Inhibition
20	0.274±0.004	86.19	0.439± 0.019	77.88
40	0.266±0.008	86.59	0.391± 0.011	80.3
60	0.234±0.007	88.21	0.351±0.006	82.22
80	0.227±0.004	86.04	0.295±0.003	85.13
100	0.185±0.004	90.68	0.253±0.004	87.25
120	0.177±0.005	91.08	0.251± 0.003	87.35
140	0.174±0.002	91.23	0.235± 0.003	88.16
160	0.159±0.002	91.98	0.229± 0.004	88.46
180	0.128±0.003	93.55	0.228± 0.004	88.51
200	0.118±0.005	94.05	0.203± 0.005	89.77

Table: 13:Superoxide anion scavenging ability of Standard and Ethanol, Methanol and Aqueous extract of *Curcuma caesia* Roxb:

Conc (µg/ml)	EECC Absorbance	% Inhibition	MECC Absorbance	% Inhibition	AECC Absorbance	% Inhibition
20	0.491± 0.004	75.26	0.559±0.004	71.83	0.791±0.077	60.7
40	0.433± 0.004	78.18	0.539±0.011	72.84	0.620±0.092	68.76
60	0.394±0.006	80.15	0.530±0.015	73.29	0.540±0.006	72.79
80	0.350±0.007	82.36	0.522±0.009	73.7	0.538±0.005	72.89
100	0.300±0.005	84.88	0.508±0.005	74.4	0.531± 0.002	73.24
120	0.273±0.004	86.24	0.475±0.011	76.07	0.528± 0.003	73.4
140	0.270±0.005	86.39	0.474±0.017	76.12	0.520± 0.004	73.8
160	0.250±0.004	87.4	0.445±0.025	77.58	0.507±0.004	74.45
180	0.234±0.004	88.21	0.400±0.118	79.84	0.499±0.009	74.36
200	0.231±0.004	88.36	0.317±0.004	84.03	0.487±0.009	75.46

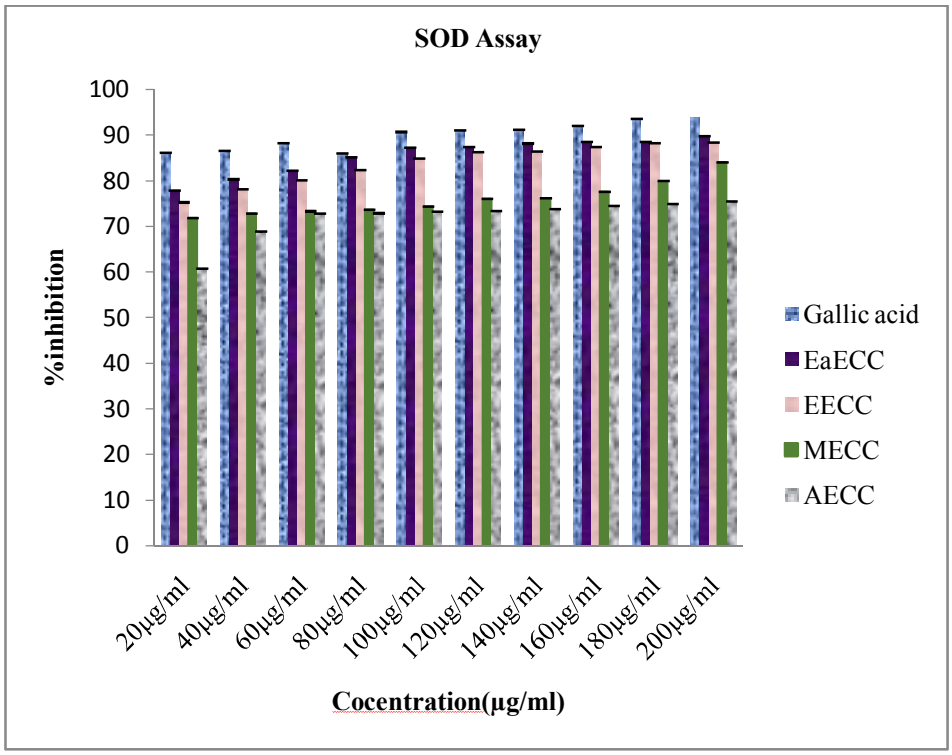


Fig: 20: Superoxide anion scavenging assay of the *C.caesia* rhizome extracts.

4.6: Antimutagenicty Assay:

Based on the promising antioxidant activity, ethyl acetate, ethanolic, methanolic and aqueous extracts were evaluated for their antimutagenic activity employing Ames test against indirect acting mutagen Cyclophosphamide. All the extracts were found to inhibit the mutagen cyclophosphamide in dose dependent manner. Linear relationship between ethyl acetate extract at different concentrations and its antimutagenic response without S9 is strong in case of TA98 (R^2 = 0.94) followed by TA 100 (R^2= 0.69) (table no.14, fig: 21 and 22). Moreover, the antimutagenic response of ethyl acetate extract of *C.caesia* Roxb was strong in both the strains TA98 (R^2 = 0.99; $p< 0.01$) and TA100 (R^2 = 0.99; $p<0.02$) in the presence of S9. The percentage inhibition of EaECC starts from 78.26% to 93.07 and it was found to be significantly different ($p< 0.02$) as compared to positive control (table no. 14, fig: 23 and 24).

Table: 14: Number of his+ revertants in *Salmonella typhimurium* strains produced by *Curcuma caesia* Roxb. extracts against Cyclophosphamide:

TA 98		TA 100					
Treatment	Conc(µg/ml)	-S9	+S9	% inhibition	-S9	+S9	% inhibition
SR		92.66±6.94	304±23		55±4.54	213±13.06	
PC	500	299.66±26.44	718±94		135.66±19.66	652.66±71.2	
EaECC	50	108.66±20.53	394±71.89*	78.26	94.66±16.21	388.88±59.72#	59.99
	500	106±26.99	384.33±44.85*	80.59	79.33±28.76	379.33±60.38#	62.16
	5000	100.33±18.78	332.66±89.13*	93.07	70±0.81	333.66±91.33#	72.55
EECC	50	116.33±18.14	395.11±71.2*	77.99	98±10.42	398.66±64.8*	57.77
	500	111.33±19.14	386.51±74*	80.07	80.33±10.63	379.33±63.8*	62.16
	5000	104.33±21.14	341.45±93.72*	90.95	71±16.63	334.66±86.51*	72.32
MECC	50	179.66±39.98	491.67±98.28*	54.66	126.66±3.39	434.33±44.93*	49.65
	500	163.66±47.6	487.66±56*	55.63	105.66±5.39	412.33±43.4*	54.66
	5000	144.66±13.55	401.66±93.14	76.41	91.66±10.17	385.33±74*	60.80

			*				
AECC	50	221.66±15.9	596.67	29.30	131.33 ±3.86	466.33±21.7 *	42.38
	500	211.33±22.8 9	562.67 ±3.5**	37.51	121±5. 54	449.66±45.7 *	46.17
	5000	95.66±31.56	479.66 ±33.15 *	57.57	97.66± 9..74	410.66±51.8 *	55.04

The data represented in the table is the Mean ± S.D values of three replicates.

*p< 0.01, **p< 0.05, #p < 0.02. EaECC: ethyl acetate extract of *C. caesia* Roxb;EECC: ethanolic extract of *Curcuma caesia* Roxb ; MECC: methanolic extract of *Curcuma caesia* Roxb., AECC: Aqueous extract of *Curcuma caesia* Roxb., PC: positive control, CP: cyclophosphamide. SR: spontaneous revertants. CP is added in all the treatment groups except in the spontaneous revertant groups. Percentage inhibition indicates the decrease in the number of revertant colonies which are made increased by the presence of mutagen cyclophosphamide in the presence of metabolic activator S9.

Linear relationship between extract at different concentrations and antimutagenic response in the case of EECC without S9 is strong in the strain TA98 (R^2 = 0.99) followed by TA100 (R^2 = 0.97) (table no.14, fig: 21and 22), with S9 it is strong in the strain TA98 (R^2 = 0.99) followed by TA100 (R^2 = 0.95) (table no. 14, fig: 23 and 24). At all the doses, the antimutagenic response was significant ($p<0.01$) against both the strains with the percent mutagenicity decrease from 77.99 to 90.95 for TA98 followed by TA100 with percent antimutagenicity starting from 57.77 to72.55. Similar trend was followed for methanoilc extract of *Curcuma caesia* Roxb. Linear relationship between extract dose and antimutagenic response in the case of MECC without S9 is strong in the strain TA98 (R^2 = 0.99) followed by TA100 (R^2 = 0.97) (table no.14, fig: 21 and 22), with S9 it is strong in the strain TA98 (R^2 = 0.99) followed by TA100 (R^2 =

0.86) (table no. 14, fig: 23 and 24). At all doses the antimutagenic response was significant at ($p<0.01$) with the percent mutagenicity decrease from 54.66 to 76.41% in case of TA98 followed by TA100 with the percent mutagenicity decrease from 49.65 to 60.80 in MECC. The significant level shown was ($p<0.01$) for all concentrations 50 µg, 500 µg and 5000µg. Linear relationship between extract dose and antimutagenic response in the case of AECC without S9 is strong in the strain TA98 ($R^2 = 0.98$) followed by TA100 ($R^2 = 0.95$) (table no.14, fig: 21 and 22), with S9 it is same for both the strain TA98 ($R^2 = 0.95$) and TA100 ($R^2 = 0.95$). At the dose of 50µg of AECC antimutagenic response was insignificant with percent inhibition of 29.30 but at 500 µg, the antimutagenic response was significant ($p<0.05$) with percent inhibition of 37.51 and at the dose 5000 µg it was significant ($p<0.01$) (57.57% inhibition) in case of TA98 and in case of TA100 in AECC the significant level shown was ($p<0.01$) for all concentrations with the percent mutagenicity decrease from 29.30 to 57.57 (table no.14, fig: 23 and 24).

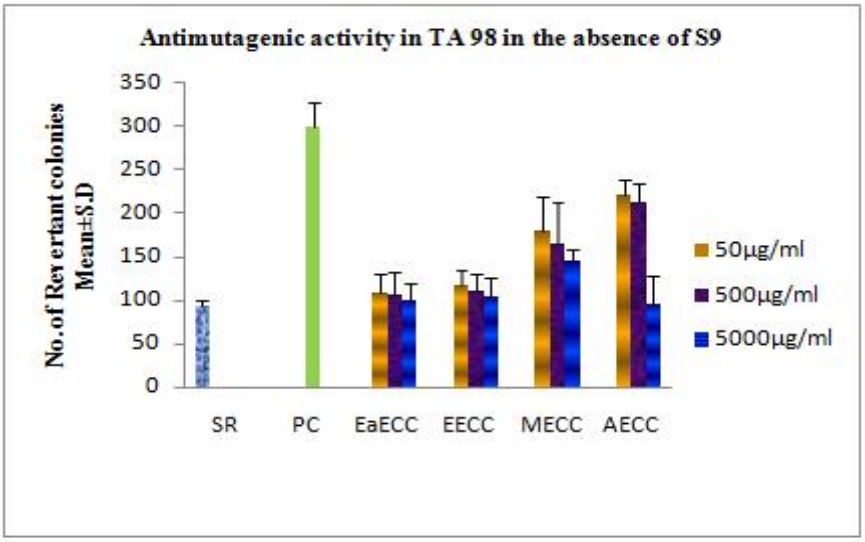


Fig: 21: Antimutagenic activity of all extracts in TA 98 in the absence of S9.

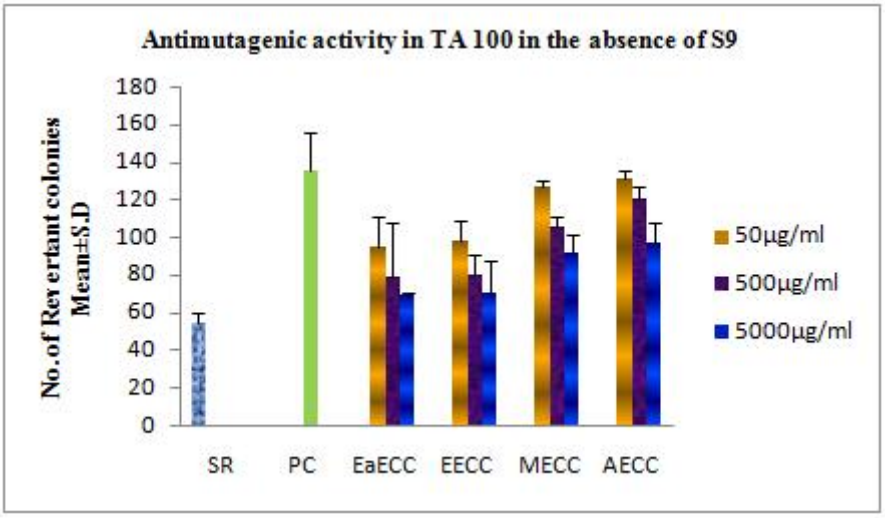


Fig: 22: Antimutagenic activity of all extracts in TA 100 in the absence of S9.

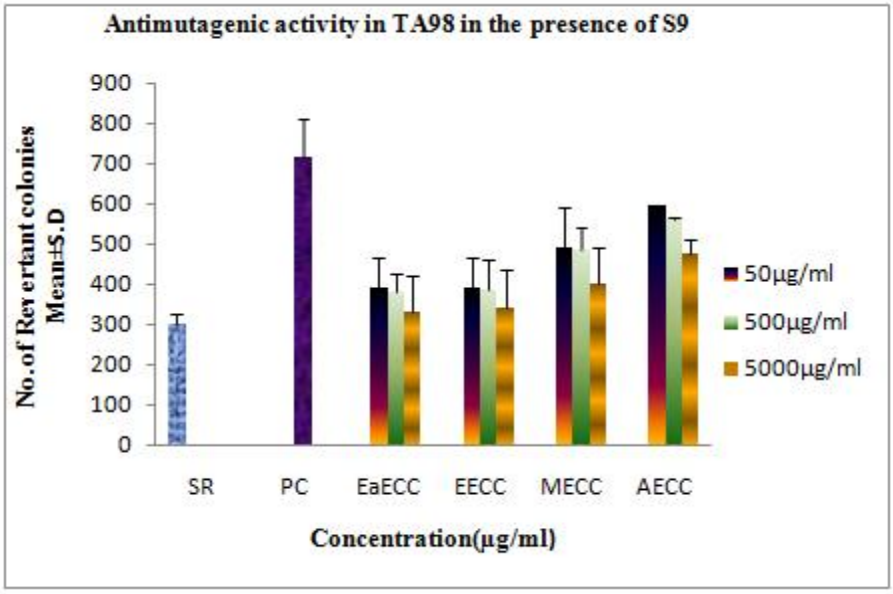


Fig: 23: Antimutagenic activity of all extracts in TA 98 in the presence of S9.

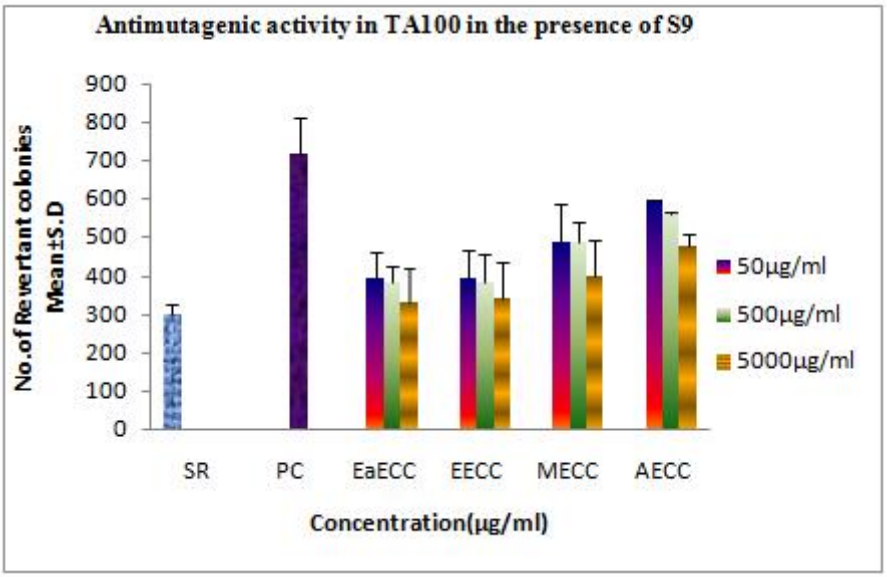
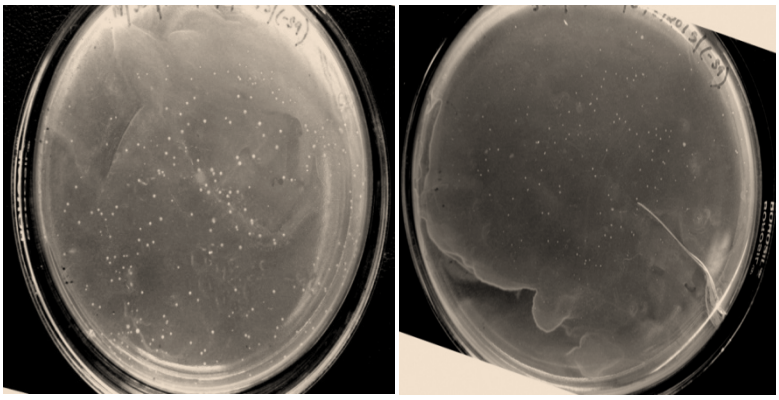
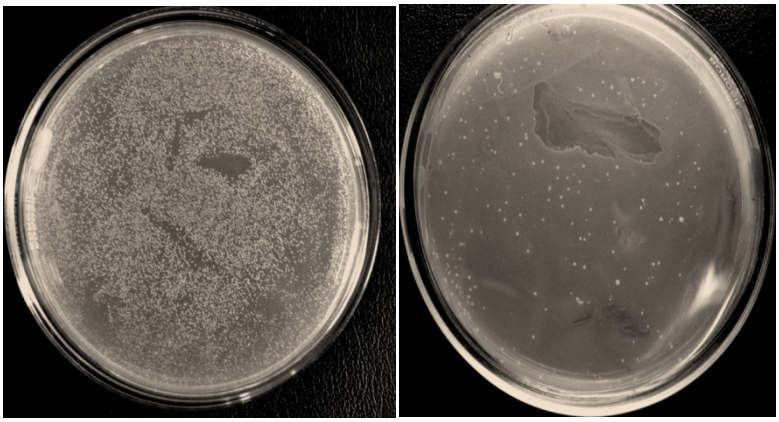


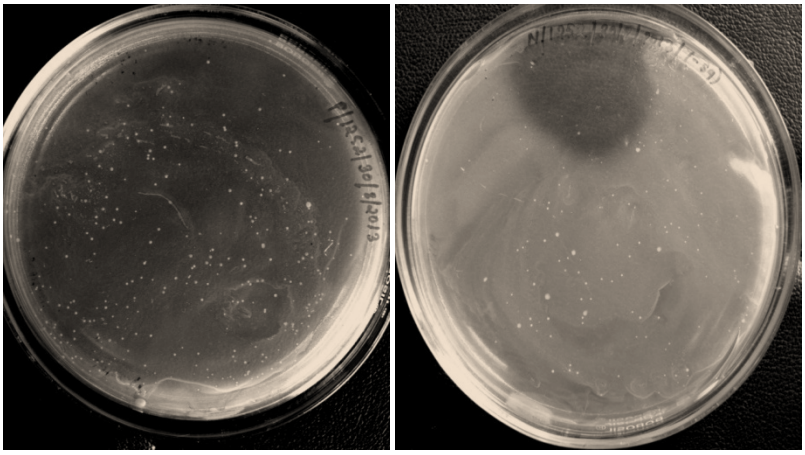
Fig: 24: Antimutagenic activity of all extracts in TA 100 in the presence of S9.



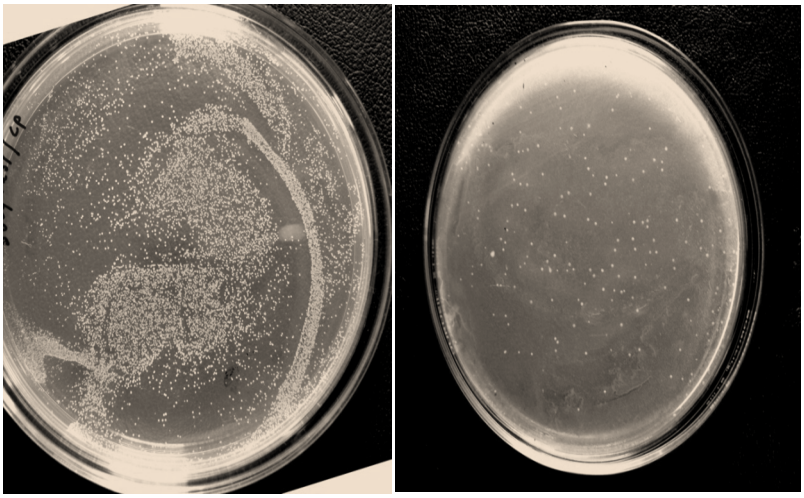
A: PC (TA 98; -S9) B: NC (TA 98; -S9)



C: PC (TA 98; +S9) D: NC (TA98; +S9)



E: PC (TA 100; -S9) F: NC (TA100; -S9)



G: PC (TA100; +S9) H: NC (TA 100; +S9)

Fig: 25: Bacterial colonies in the petriplates.

A: no. of revertant colonies in TA 98 in positive control in the absence of metabolic activator (S9); B: no. of revertant colonies in TA 98 in negative control in the absence of metabolic activator (S9); C: no. of revertant colonies in TA 98 in positive control in the presence of metabolic activator (S9); D: no. of revertant colonies in TA 98 in negative control in the presence of metabolic activator (S9); E: no. of revertant colonies in TA 100 in positive control in the absence of metabolic activator (S9); F: no. of revertant colonies in TA 100 in negative control in the absence of metabolic activator (S9); G: no. of revertant colonies in TA 100 in positive control in the presence of metabolic activator (S9); H: no. of revertant colonies in

TA 100 in negative control in the presence of metabolic activator (S9).

4.7: Acute toxicity studies:

Treatment of animals with different extracts of *Curcuma caesia* Roxb. did not show any sign of toxicity during the period of investigation. No mortality was observed even at the highest dose of 2000mg/ kg. b. wt. Animals from both the control as well *C.caesia* extracts treated groups showed normal patterns of awareness, somato motor activity, touch response and sound response. There was no sign of change in behavior, skin and fur color, no salivation, diarrhea, lethargy, tremors, sleep as well as coma (table no.15).

Table no: 15: General behavior of animals:

Behavioral Pattern	Control	*C.caesia* extracts at 2000mg/kg.b.wt
Touch response	Normal	Normal
Somato motor activity	Normal	Normal
Behavior	Normal	Normal
Skin and Fur color	No change	No change
Salivation	Absent	Absent
Diarrhea	Absent	Absent
Lethargy	Absent	Absent
Tremors	Absent	Absent
Sound response	Present	Present

Table: 16: Mortality Observation (Acute Toxicity Study):

Treatment	**Dose (mg/kg)/oral**	**Sighting study**	**14 days study (Main study)**
CCE	5	0/3	-
	50	0/3	-
	300	0/3	-
	2000	0/3	0/5
Control	-	0/3	0/5

CCEs did not show any mortality in sighting study as well as main study of acute toxicity study.

Table: 17: Body weight:

Treatment (mg/kg)	First day	1st Week	2nd Week
Control	23.906±0.850	25.158±1.573	28.526±1.806
EaECC	23.202±1.472	25.254±1.193	27.622±1.277
EECC	23.514±0.626	25.684±1.001	27.148±1.913
MECC	23.746±1.157	24.560±1.179	26.366±1.103

Each value is the mean ± S.D (n=5)

Initially the average body weight of the control untreated mice and the EaECC, EECC, MECC treated mice was 23.906±0.850 g, 23.202±1.472g, 23.514±0.626g, 23.746±1.157g respectively which increases after 1st and 2nd weeks (table no.17).

4.8: Antigenotoxicity Assay:

Damaging effect on DNA (micronuclei formation) was observed to be more in CP treated mice at the dose of 50mg/kg.b.wt (positive control group) as shown in table no.18, 19 and 20. Percentage of micronuclei formed in PC group were high (15.36%) as compared to NC groups (0.46%) significantly ($p<0.002$). However, pre-treatment of each extract at different concentrations (100, 250, 500mg/kg.b.wt of mice) reduces the DNA damage. The percentage inhibition of DNA damage: by EaECC was found to be 46% ($p<0.01$), 57.03% ($p<0.01$) and 71.87($p<0.001$); by EECC: was found to be 43.8%, 54.42% and 69% significantly ($p<0.01$ and $p<0.05$) at the concentrations of 100, 250, 500mg/kg.b.wt of mice as compared to positive control. The pre treatment of different concentrations of MECC followed by CP also reduces the micronuclei formation significantly ($p< 0.005$, $p< 0.01$, $p<0.001$). The reduction percentage of micronuclei by MECC was 41.77%, 48.43% and 68.75% at different concentrations (100, 250, 500mg/kg.b.wt respectively). There was no sign of genotoxicity in the treatment with extracts only, since the values were almost near to normal

groups and they are not significantly different from the negative control groups (Table 18, 19 and 20).

Table: 18:The effect of treatment with EaECC on the micronuclei induced by CP in bone marrow cells of mice:

Treatment	No. of cells analysed	MNPCEs	% frequency	Reduction %
NC	1000	4.6± 2.65	0.46	
PC	1000	153.6±46.79***	15.36	
Solution 1	1000	2.2± 1.6	0.22	
Solution 2	1000	3.2± 3.18	0.32	
Solution 3	1000	5± 3.40	0.5	
Solution 1+ CP	1000	82.25± 1.92*	8.2	46.45
Solution 2+ CP	1000	66± 32.829*	6.6	57.03
Solution 3+ CP	1000	43.2± 5.49**	4.32	71.87

*p<0.01 ; **p<0.001; ***p<0.0002 (One way Anova).

Solution 1:100mg of the extract per kg. b. wt; Solution 2: 250mg of the extract per kg. b. wt; Solution3: 500mg of the extract per kg. b. wt; CP: cyclophosphamide; NC: Negative Control; PC: Positive Control.

Table: 19:The effect of treatment with EECC on the micronuclei induced by CP in bone marrow cells of mice:

Treatment	No. of cells analysed	MNPCEs	% frequency	Reduction %
NC	1000	4.6± 2.65	0.46	
PC	1000	153.6±46.79***	15.36	
Solution 1	1000	3.6± 3.00	0.36	
Solution 2	1000	4.2± 2.13	0.42	
Solution 3	1000	6.8± 3.12	0.68	
Solution 1+ CP	1000	86.2± 37.35**	8.62	43.8
Solution 2+ CP	1000	70± 31.74*	7	54.42
Solution 3+ CP	1000	47.6± 3.49*	4.76	69

*p<0.01; **p<0.05; *** p<0.002 (One Way Anova).

Solution 1:100mg of the extract per kg. b. wt; Solution 2: 250mg of the extract per kg. b. wt; Solution3: 500mg of the extract per kg. b. wt; CP: cyclophosphamide; NC: Negative Control; PC: Positive Control.

Table: 20:The effect of treatment with MECC on the micronuclei induced by CP in bone marrow cells of mice:

Treatment	No. of cells analysed	MNPCEs	% frequency	Reduction %
NC	1000	4.6± 2.65	0.46	
PC	1000	153.6±46.79***	15.36	
Solution 1	1000	4.4 ± 4.07	0.44	
Solution 2	1000	5.4± 1.01	0.54	
Solution 3	1000	8.4± 2.15	0.84	
Solution 1+ CP	1000	89.4± 31.79**	8.94	41.77
Solution 2+ CP	1000	79.2± 21.18*	7.92	48.43
Solution 3+ CP	1000	48± 2.09#	4.8	68.75

*p<0.01; **p<0.005; #p<0.001; ***p<0.002 (One way Anova).

Solution 1:100mg of the extract per kg. b. wt; Solution 2: 250mg of the extract per kg. b. wt; Solution3: 500mg of the extract per kg. b. wt; CP: cyclophosphamide; NC: Negative Control; PC: Positive Control.

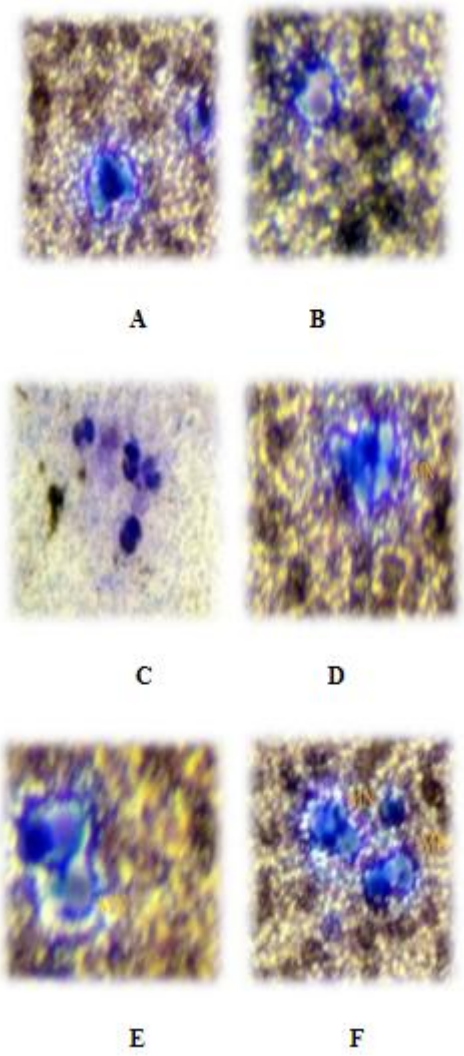


Fig: 26: Micronuclei and Normal cells. A and B: cells with normal nuclei; C: cell with binucleated nuclei; D: micronuclei formation in binucleated cell; E and F: micronuclei formation.

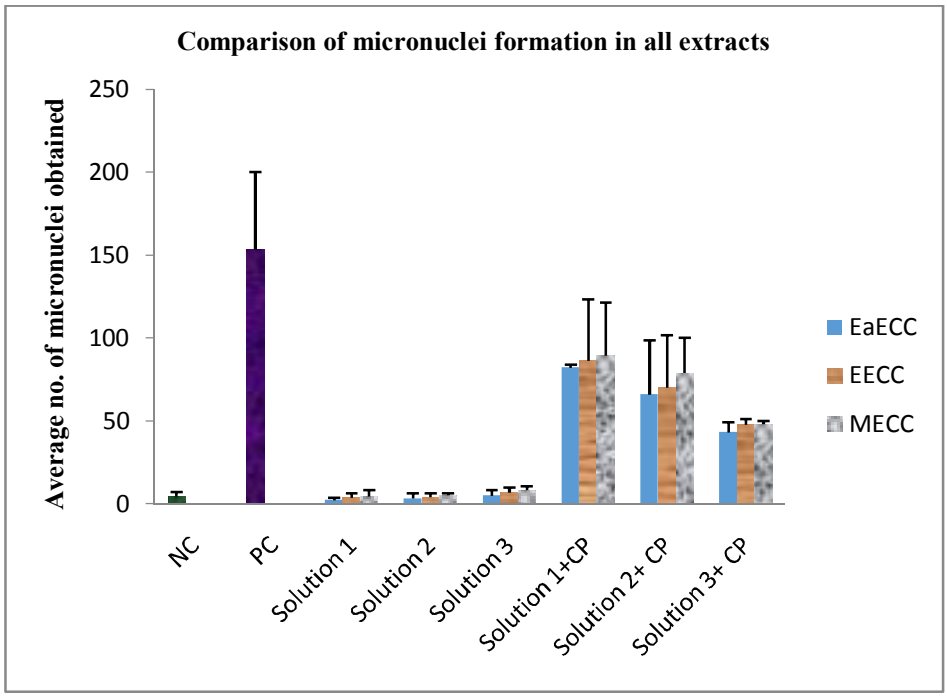


Fig: 27: Comparison of micronuclei formation in all extracts.

Solution 1:100mg of the extract per kg. b. wt; Solution 2: 250mg of the extract per kg. b. wt; Solution3: 500mg of the extract per kg. b. wt; CP: cyclophosphamide; NC: Negative Control; PC: Positive Control.

Fig: 27, shows that treatment of *Curcuma caesia* Roxb only did not show any toxicity to the mice since they are not significantly different from the negative control groups. Positive control groups showed formation of high number of micronunuclei as compare to negative groups. However pretreatment of all extracts for seven consecutive days found to inhibit the formation of micronuclei significantly as compared to positive control groups (fig:27).

4.9: Biochemical Analysis:

4.9.1: SGOT and SGPT Analysis:

The significance level of the positive control group was compared to the negative control group and it was found statistically

significant at $p<0.01$ in SGOT analysis and $p<0.0001$ in case of SGPT analysis. Statistical values of other groups were compared to positive control groups as shown in table no. 21.

The increased levels of SGOT and SGPT in the serum resulting from the leakage from liver have been marked by many researchers as an indication of toxicity to the liver. The present study shows that the levels of SGOT (444U/ml) and SGPT (101.33U/ml) were statistically higher in positive control groups as compared to negative control groups (43U/ml in SGOT and 6U/ml in SGPT) indicating toxicity to the liver as shown in table no.21. But the pre-treatment of each extract at different concentrations (100, 250 and 500mg/kg body wt) reduces the leakage of SGOT and SGPT from the liver indicating the protective effect of the extract (table no.21)

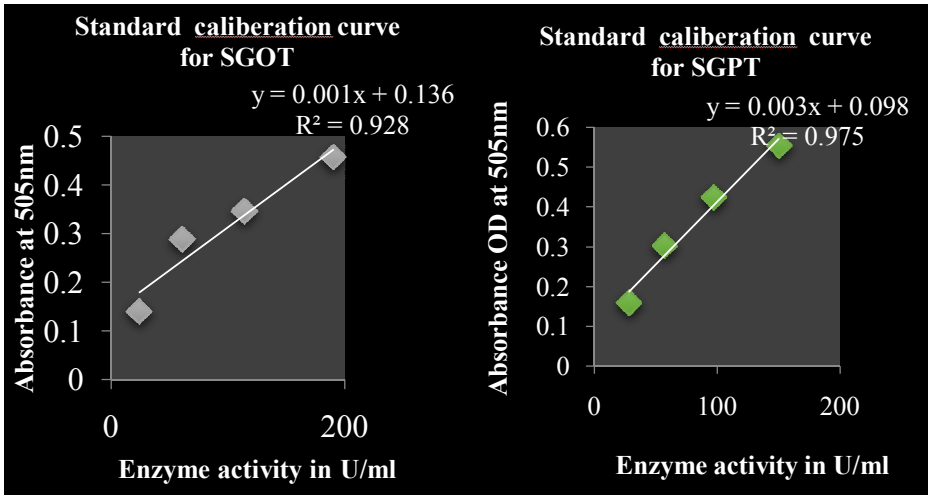


Fig: 28: Standard calibration curve for SGOT and SGPT.

Table: 21: SGOT and SGPT analysis:

Treatment	SGOT(EaECC)	SGOT(EECC)	SGOT(MECC)	SGPT(EaECC)	SGPT(EECC)	SGPT(MECC)
NC	43±0.244	43±0.244	43±0.244	6±0.116	6±0.116	6±0.116
Solution 1 +CP	228±0.113**	278±0.087***	286±0.095*	30.33±0.036#	32±0.041****	33.67±0.036 #
Solution 2+CP	161±0.113**	181±0.093****	185±0.175*	17±0.08****	22±0.048****	23.33±0.082**
Solution 3+CP	52±0.162***	99±0.151**	108±0.144 **	13±0.061#	13.33±0.083#	18±0.152***
PC	444±0.017*	444±0.017*	444±0.017*	101.33±0.041#	101.33±0.041#	101.33±0.041#
Solution 3	41± 0.243	44± 0.244	46± 0.244	5± 0.074	6± 0.073	6.33± 0.074

* p< 0.01; ** p< 0.001; *** p< 0.005; **** p< 0.0005; # p< 0.0001

4.9.2: Lipid Peroxidation and GSH Assay in the liver:

Treatment of CP (50mg/kg.b.wt) cause toxicity to the liver, which was indicated by the increased lipid per oxidation as shown in table no. 22 and figure no.29. In positive control groups lipid peroxidation form was more i.e., 0.550 nmole of TBARS/ g tissue (p<0.005) as compared to negative control group (0.120 nmole TBARS/g tissue) which is significantly different and the level of GSH in the positive control groups was found to be reduced 0.007 nmole of GSH/g tissue; significantly (p<0.0001) as compared to negative control

groups with the value of 0.182 nmole of GSH/g tissue. Moreover the pretreatment of all the extracts studied showed inhibition of lipid peroxidation in the order of EaECC> EECC> MECC as well as increases the level of GSH with the pretreatment of all the extracts as shown in table no.22 and fig: 30.

Table: 22: Lipid Peroxidation and GSH Assay in the liver:

Treatment	LPO(L) EaECC	LPO(L) EECC	LPO(L) MECC	GSH(L) EaECC	GSH(L) EECC	GSH(L) MECC
NC	0.120±0.047	0.120±0.047	0.120±0.047	0.182±0.052	0.182±0.052	0.182±0.052
Solution 1+ CP	0.253±0.076a	0.260±0.061a	0.267±0.036a	0.078±0.043*	0.072±0.043*	0.060±0.047a
Solution 2 +CP	0.192±0.037b	0.240±0.091a	0.251±0.087a	0.096±0.058*	0.091±0.057*	0.060±0.047a
Solution 3 + CP	0.176±0.008a	0.179±0.010*	0.179±0.010*	0.119±0.058***	0.115±0.056***	0.111±0.054***
PC	0.550±0.255***	0.550±0.255***	0.550±0.255***	0.007±0.003#	0.007±0.003#	0.007±0.003#
Solution 3	0.117±0.043	0.117±0.044	0.118±0.046	0.189±0.065	0.187±0.066	0.185±0.068

*** p< 0.005; a p< 0.05 ; b p< 0.02 ;* p< 0.01 ; # p< 0.0001; ** p< 0.001 (One way Anova).

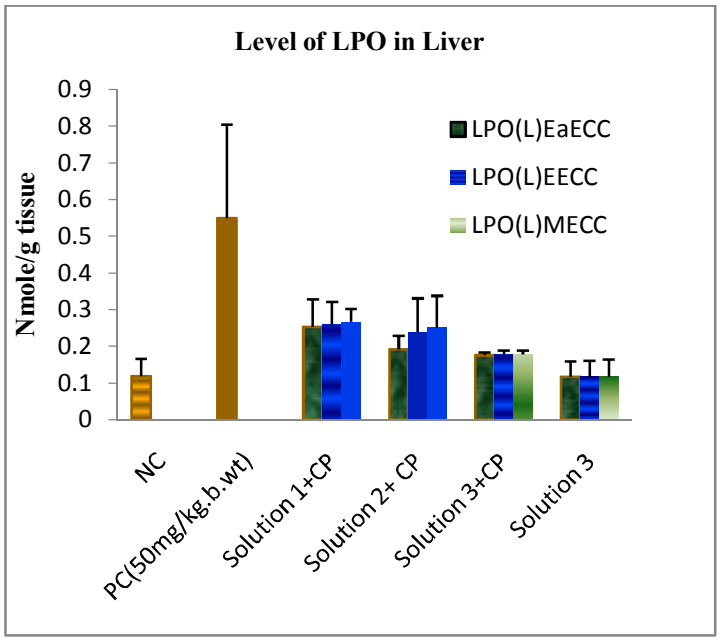


Fig: 29: Lipid peroxidation in the liver of CP and extract treated mice.

Solution 1:100mg of the extract per kg. b. wt; Solution 2: 250mg of the extract per kg. b. wt; Solution3: 500mg of the extract per kg. b. wt; CP: cyclophosphamide; NC: Negative Control; PC: Positive Control.

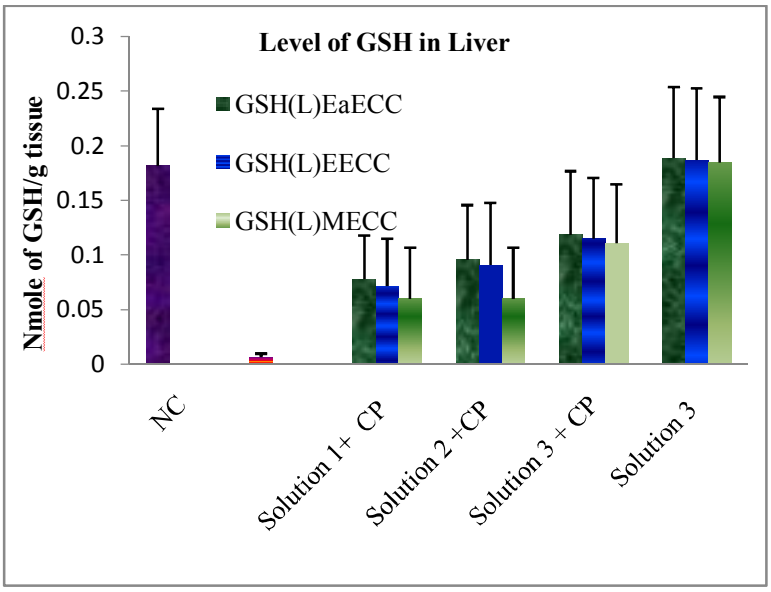


Fig: 30: Level of GSH in the liver of CP treated and extract treated mice.

4.9.3: GR and Protein content in the liver of mice:

Besides the lipid peroxidation and decreasing level of GSH caused by CP in the positive control in the liver, the amount of GR and protein were also found to be reduced in the liver significantly as compared to negative control groups. The level of GR in the liver of CP treated group was found to be 0.048 µmoles of NADPH oxidized/min/g tissue while the negative control group was found to be 1.920 µmoles of NADPH oxidized/min/g tissue. It was found to be significantly different ($p<0.0001$) (Table no.23 and Fig: 31). The levels of protein oxidized in the CP treated group were also higher as compare to the negative control group. The total protein thiol content in the negative control group was found to be 22.58 mg/g wt of tissue as compared to the positive control group with the value of -13.75 mg/g wt of tissue which is significantly different as compared to the normal group ($p< 0.05$) (Table 23 and Fig:32).

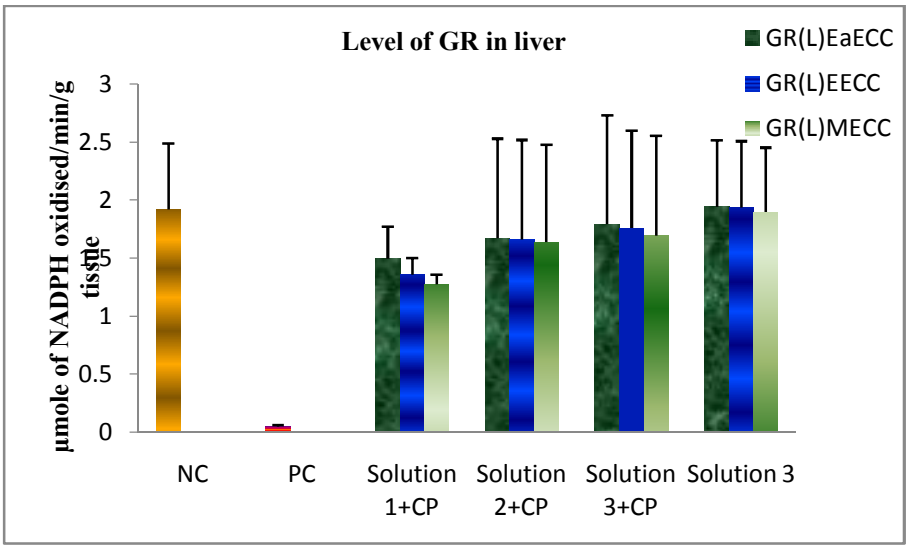


Fig: 31: Level of GR in liver.

Table: 23: Level of GR and protein in the liver of CP treated and extract treated mice:

Treatment	GR(L) EaECC	GR(L) EECC	GR(L) MECC	Protein(L) EaECC	Protein(L) EECC	Protein(L) MECC
NC	1.920±0.571	1.920±0.571	1.920±0.571	22.58±0.085	22.58±0.085	22.58±0.085
Solution 1+ CP	1.496±0.276a	1.359±0.144a	1.274±0.084a	4.58±0.068***	-1.833±0.082a	-3.33±0.065a
Solution 2 +CP	1.670±0.861***	1.661±0.859***	1.641±0.837***	11.33±0.010#	8.66±0.156*	6.25±0.085***
Solution 3 + CP	1.792±0.940***	1.759±0.841*	1.695±0.861***	19.75±0.136**	15.91±0.059#	14.16±0.113**
PC	0.048±0.015#	0.048±0.015#	0.048±0.015#	-13.75±0.092a	-13.75±0.092a	-13.75±0.092a
Solution 3	1.945±0.572	1.939±0.570	1.897±0.556	23±0.082	23±0.086	22.91±0.085

*** p< 0.005; a p< 0.05 ; b p< 0.02 ;* p< 0.01 ; # p< 0.0001; ** p< 0.001 (One way Anova)

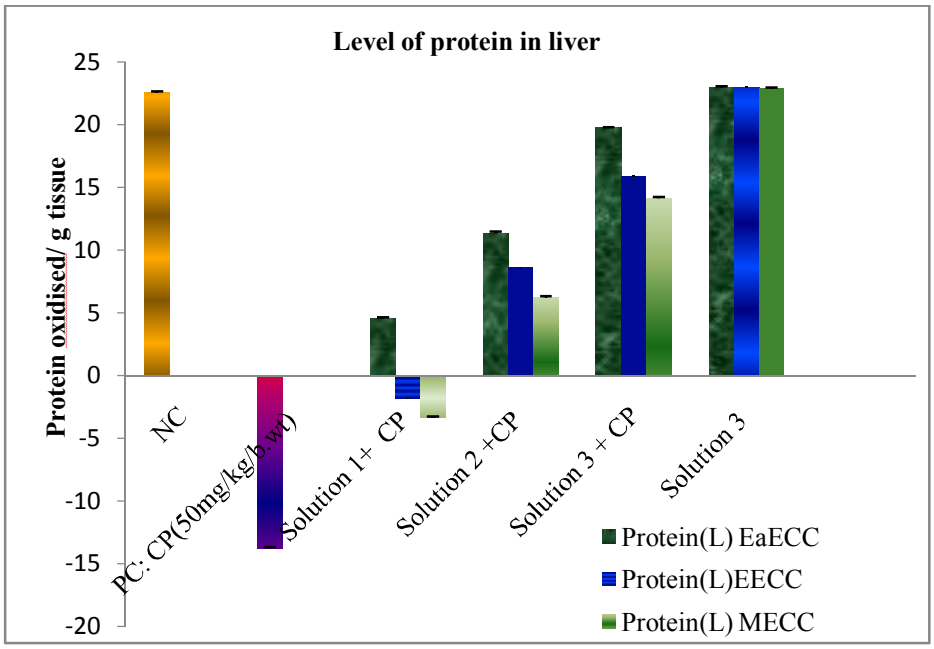


Fig: 32: Level of protein in liver.

However the pretreatment of all the extracts before the administration of CP increases the level of both GR and protein in liver significantly as compared to positive control groups (Table no.23, fig: 31 and 32).

4.9.4: Lipid Peroxidation and GSH Assay in the Kidney of mice:

Like the liver, the reactive metabolites of CP also affect the lipid membrane of kidney and GSH. Peroxidation level of kidney in the positive control group was found to be 0.936 nmole of TBARS/ g tissue, which is significantly high ($p<0.02$) as compared to the negative control group with the value of 0.236 nmole of TBARS/ g tissue. Likewise, value of GSH in the positive control group was found to be 0.011 nmole of GSH/g tissue which is significantly reduced ($p<0.05$) from the negative control group 0.082 nmole of GSH/g tissue (table no.24, fig: 33 and 34). This indicates that the reactive metabolites produced by CP also affect the kidney.

Table: 24: Level of LPO and GSH in the kidney of CP treated and extract treated mice:

Treatment	LPO(K) EaECC	LPO(K) EECC	LPO(K) MECC	GSH(K) EaECC	GSH(K) EECC	GSH(K) MECC
NC	0.236±0.183	0.236±0.183	0.236±0.183	0.082±0.063	0.082±0.063	0.082±0.063
Solution 1+ CP	0.318±0.116**	0.347±0.086***	0.352±0.083***	0.045±0.021*	0.039±0.009****	0.025±0.006***
Solution 2 +CP	0.249±0.148**	0.309±0.075c	0.313±0.075c	0.058±0.024***	0.057±0.029*	0.047±0.022*
Solution 3 + CP	0.170±0.212**	0.227±0.165c	0.237±0.166c	0.083±0.063a	0.079±0.027**	0.065±0.033*
PC	0.936±0.254b	0.936±0.254b	0.936±0.254b	0.011±0.004a	0.011±0.004a	0.011±0.004a
Solution 3	0.220±0.042	0.221±0.043	0.236±0.082	0.095±0.056	0.075±0.053	0.072±0.056

** p< 0.001; b p< 0.02 ; *** p< 0.005; c p< 0.001 ; a p< 0.05 ;* p< 0.01; **** p< 0.0005 (One way Anova).

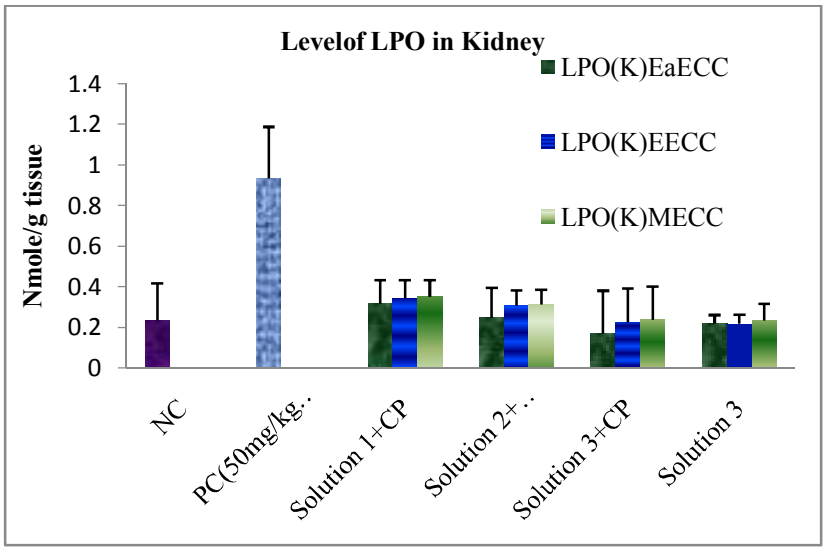


Fig: 33: Level of LPO in the kidney of CP treated and extract treated mice.

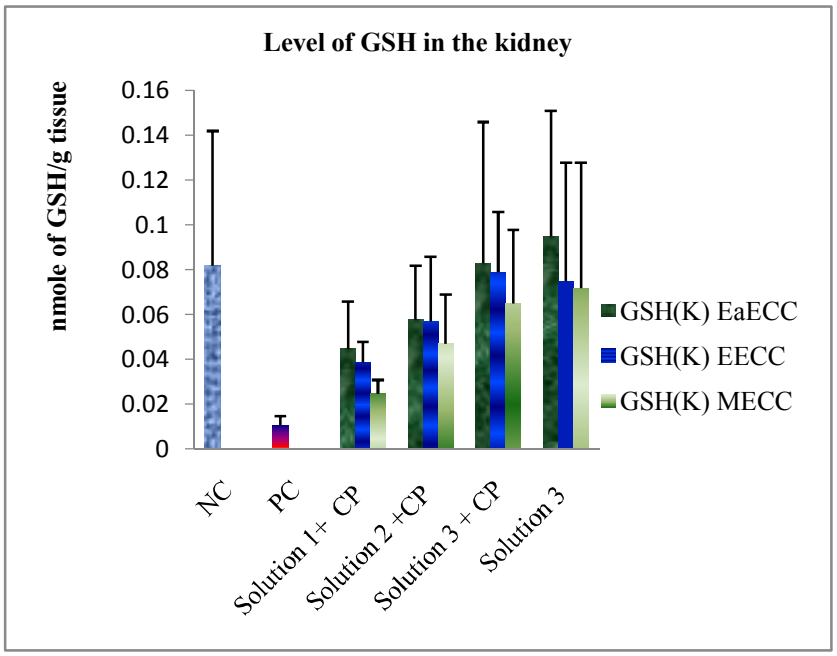


Fig: 34: Level of GSH in the kidney of CP treated and extract treated mice.

The extracts of *Curcuma caesia* Roxb. studied is so effective that the pretreatment of all the extracts before administration of CP in mice reduces the level of peroxidation caused by CP in the kidney and

increases the value of non enzymatic antioxidant glutathione (GSH)(table no. 24).

4.9.5: GR and Protein Content in the kidney of mice:

Cyclophosphamide toxicity in the kidney also reduces the enzymatic antioxidant level of GR and the total protein concentration in the kidney. The level of GR in the positive control group of mice was found to be 0.851 µmoles of NADPH oxidized/min/g tissue as compared to negative control group of mice with the value of 2.148 µmoles of NADPH oxidized/min/g tissue which is significantly different ($p<0.005$) (Table no.25 and fig:35). The total concentration of protein was also found to be reduced in the positive control group with the value of -7.33 µg from 20.75 µg in negative control group which is significantly different($p<0.001$) (Table no.25 and fig:36).

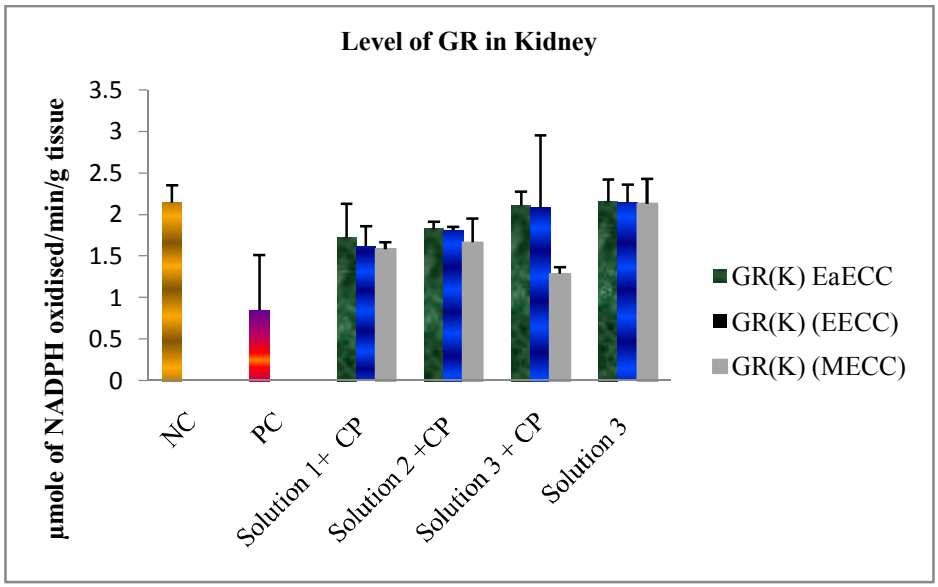


Fig: 35: Level of GR in the kidney of CP treated and extract treated mice.

Table: 25: Level of GR and protein in the kidney of CP and extracts treated mice:

Treatment	GR(K) EaECC	GR(K) (EECC)	GR(K) (MECC)	Protein(K) EaECC	Protein(K) EECC	Protein(K) MECC
NC	2.148±0.217	2.148±0.217	2.148±0.217	20.75±0.085	20.75±0.085	20.75±0.085
Solution 1+ CP	1.724±0410 a	1.627±0.238a	1.588±0.083a	3±0.038a	2.58±0.008a	-6.33±0.003a
Solution 2 +CP	1.834±0.079 *	1.817±0.038*	1.667±0.290a	7.58±0.051*	6.83±0.037*	4.833±0.079a
Solution 3 + CP	2.118±0.162 ***	2.083±0.875a	1.291±0.076*	16.91±0.055**	13.91±0.084***	12.25±0.110*
PC	0.851±0.663 ***	0.851±0.663***	0.851±0.663***	-7.33±0.105**	-7.33±0.105**	-7.33±0.105**
Solution 3	2.154±0.273	2.149±0.215	2.133±0.299	21.08±0.085	21±0.085	20.83±0.086

** p< 0.001; [b] p< 0.02 ; *** p< 0.005; [c] p< 0.001 ; [a] p< 0.05 ;* p< 0.01; **** p< 0.0005 (One way Anova).

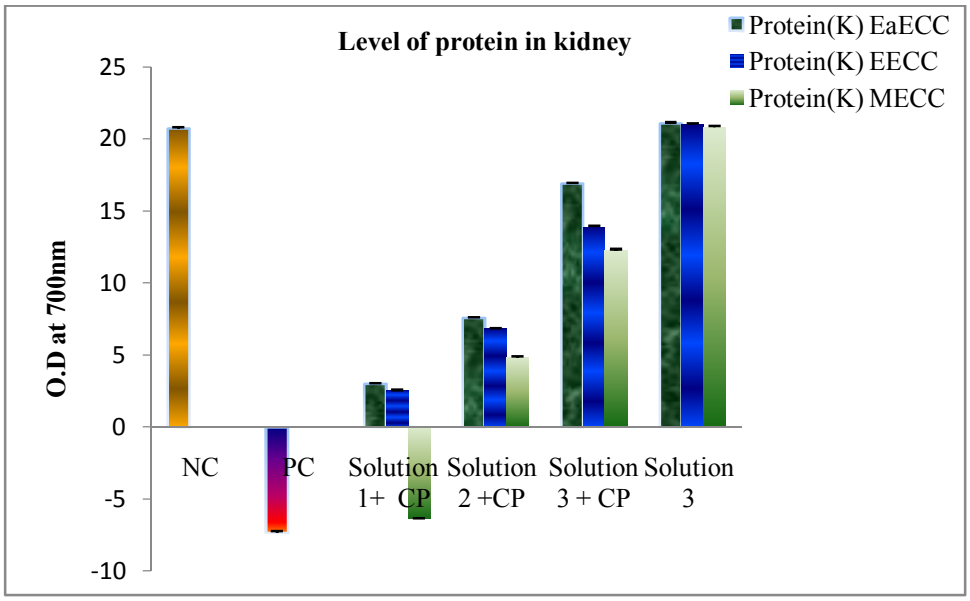


Fig: 36: Level of protein in the kidney of CP treated and extract treated mice.

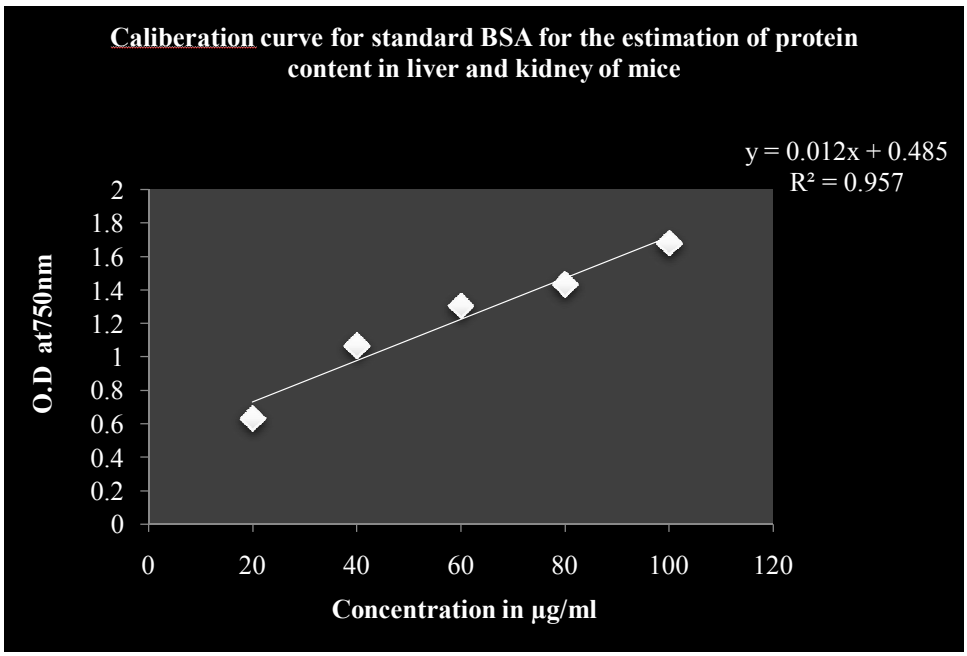


Fig: 37: Caliberation curve for standard BSA for the estimation of protein content in liver and kidney.

However the pretreatment of all extracts studied protects the changes brought about by cyclophosphamide in kidney as shown in table no.24, 25 and fig: 33, 34, 35 and 36.

4.10: Histopathological Analysis:

4.10.1: Histopathological Analysis of Liver:

Histopathological analysis of liver reveals that the liver of the negative control group of animals showed regular cellular architechture with distinct hepatic cells, sinusoidal spaces and clear central vein (fig: 38, 39 and 40: A). On the other hand, section of liver from the mice treated with CP alone (grp II) showed inflammation in the central vein and decrease in sinusoidal spaces (B of grp II in the fig: 38, 39 and 40). The pretreatment of all the extracts EaECC, EECC and MECC at different concentrations 100mg, 250mg and 500mg (grp III, IV and V) before the administration of CP (50mg/kg.b.wt) revealed a better presentation of normal architechture of liver (Fig: 38, 39, 40: C, D, E, respectively). Each extract at the dose of 500mg/kg.b.wt helps in generation of normal architecture of the liver as compare to other lower dose groups. The treatment of mice with highest concentration 500mg/kg.b.wt of each extract only showed normal liver architecture (fig: 38: F; fig: 39: F; fig: 40: F).

Histopathological Analysis of Liver:

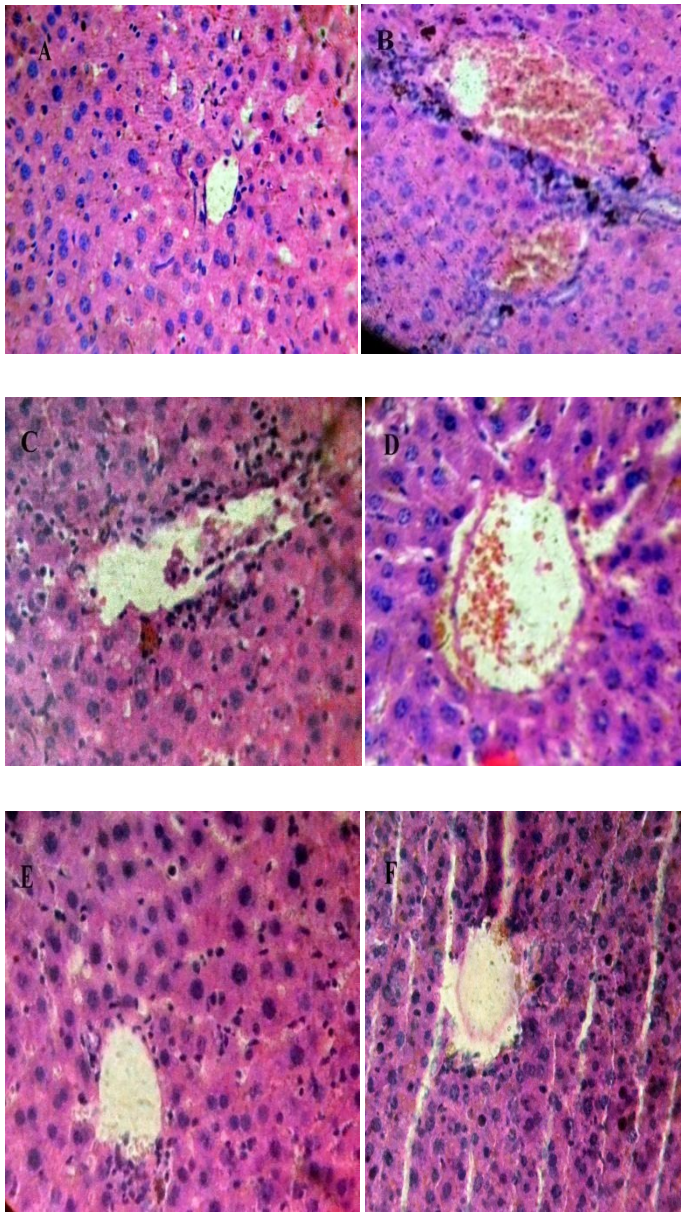


Fig: 38: A: showed regular cellular architecture with distinct hepatic cells, sinusoidal space and central vein; B: showed inflammation in the central vein of positive control group. The treatment of animals with CP+EaECC at 100, 250 and 500mg/kg.b.wt and EaECC at 500mg (c-f) revealed a better preservation of normal liver architecture. The observation is in agreement with the observation of Swarnlata *et al*., 2014.

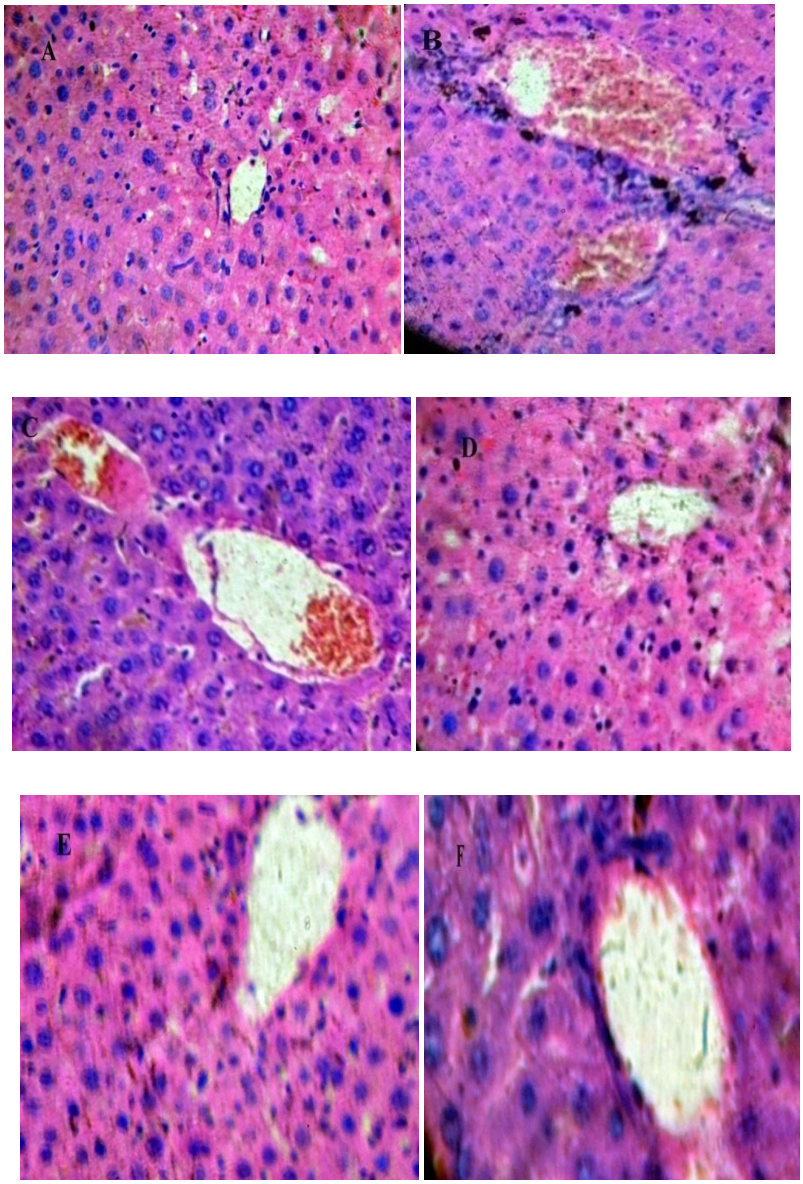


Fig: 39: A: showed regular cellular architecture with distinct hepatic cells, sinusoidal space and central vein; B: showed inflammation in the central vein of positive control group. The treatment of animals with CP+EECC at 100, 250 and 500mg/kg.b.wt and EECC at 500mg (c-f) revealed a better preservation of normal liver architecture. The observation is in agreement with the observation of Swarnlata *et al*., 2014.

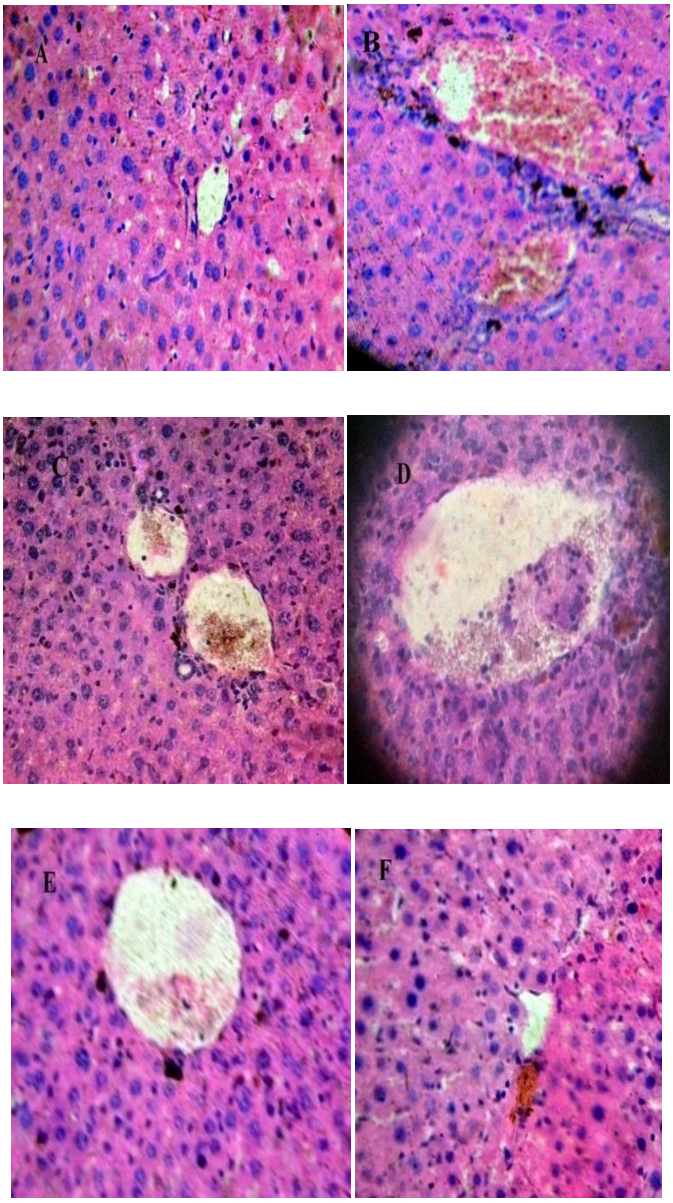


Fig: 40: A: showed regular cellular architecture with distinct hepatic cells, sinusoidal space and central vein; B: showed inflammation in the central vein of positive control group. The treatment of animals with CP+MECC at 100, 250 and 500mg/kg.b.wt and MECC at 500mg (c-f) revealed a better preservation of normal liver architecture. The observation is in agreement with the observation of Swarnlata *et al*., 2014.

4.10.2: Histopathological analysis of kidney:

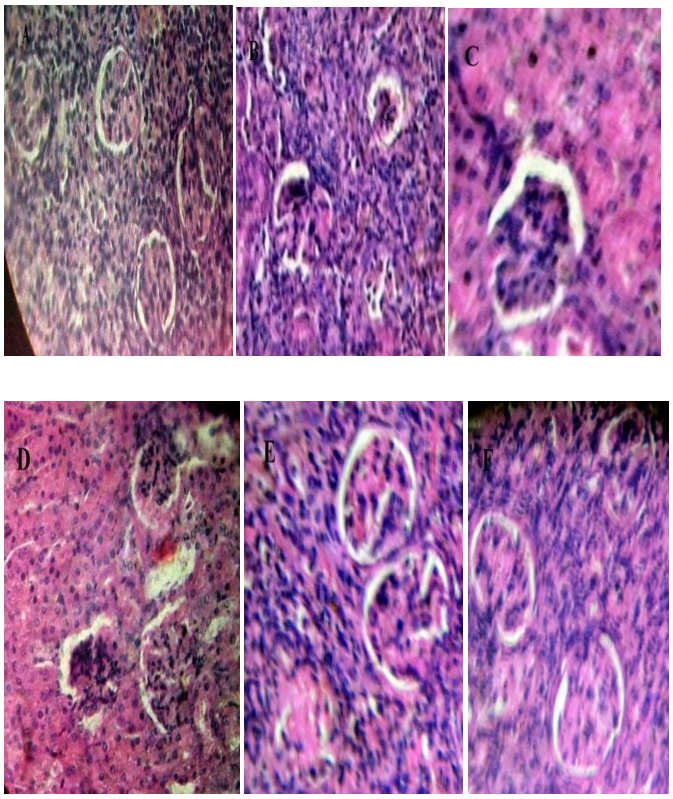


Fig: 41: A: Kidney section from normal group shows normal renal histoarchitecture .

B: kidney section from only CP treated group shows loss of normal renal architecture, inflammatory cell infiltration and fatty changes with swelling; a kidney section of CP+EaECC treated at lower dose 100mg/kg.b.wt C); at 250 mg/ kg.b.wt (D); at 500 mg /kg.b.wt (E). F: shows treatment with 500mg/kg.b.wt of EaECC only. The observation of the morphology of kidney section is in agreement with the observation of Muneeb *et al*., 2012.

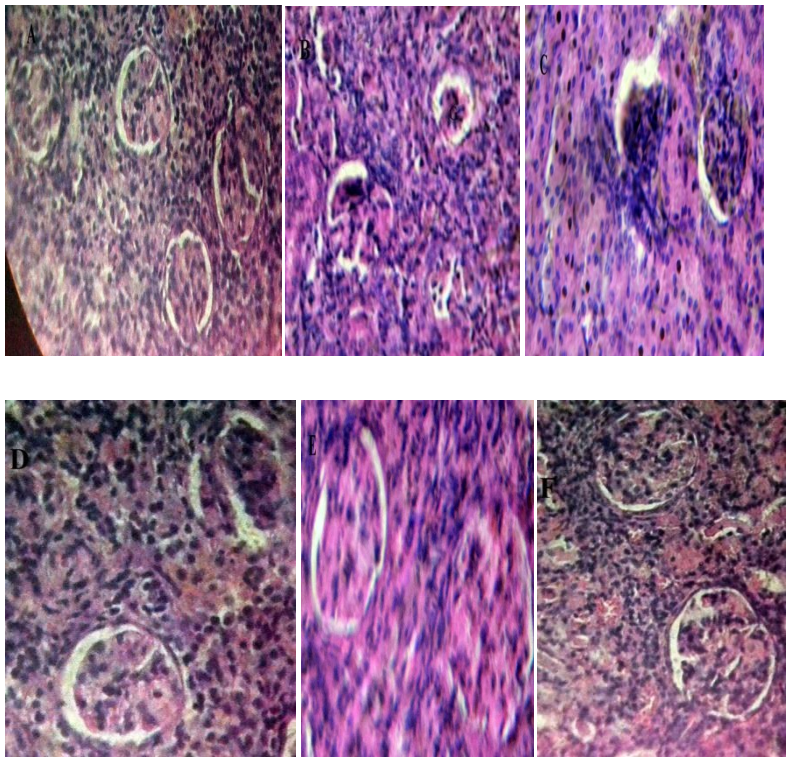


Fig: 42:A: Kidney section from normal group shows normal renal histoarchitecture B: kidney section from only CP treated group shows loss of normal renal architecture, inflammatory cell infiltration and fatty changes with swelling; a kidney section of CP+EECC treated at lower dose 100mg/kg.b.wt(C); at 250 mg/ kg.b.wt (D); at 500 mg /kg.b.wt (E). F: shows treatment with 500mg/kg.b.wt of EECC only. The observation of the morphology of kidney section is in agreement with the observation of Muneeb *et al.*, 2012 .

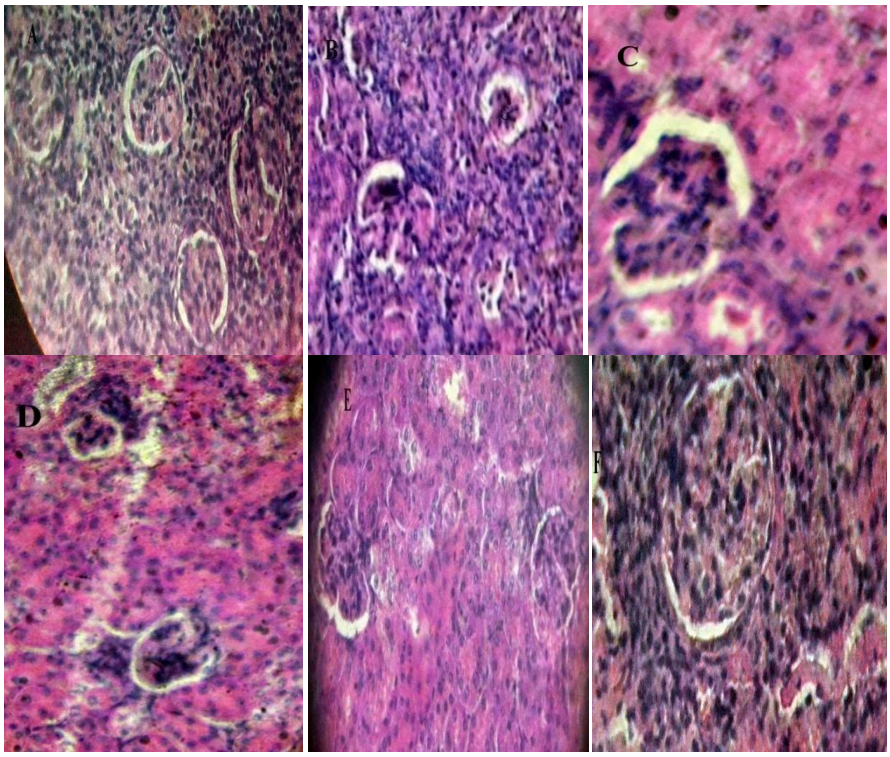


Fig: 43:A: Kidney section from normal group shows normal renal histoarchitecture B: kidney section from only CP treated group shows loss of normal renal architecture, inflammatory cell infiltration and fatty changes with swelling; a kidney section of CP+MECC treated at lower dose 100mg/kg.b.wt (C); at 250 mg/ kg.b.wt (D); at 500 mg /kg.b.wt (E). F: shows treatment with 500mg/kg.b.wt of MECC only. The observation of the morphology of kidney section is in agreement with the observation of Muneeb *et al.*, 2012.

4.11: Cytotoxicty Assay of the extracts against cancer cell lines:

4.11.1: Cytotoxicity activity of the extracts against breast cancer cell lines MDAMB231:

The antiproliferation activity was tested using human breast cancer cell lines MDAMB231 and lung cancer cell lines Calu6. According to MTT assay (table no.26 and fig: 44), it was found that

EaECC shows antiproliferating activity against breast cancer cell lines MDAMB 231 and lung cancer cell lines Calu6 with percentage inhibition of 79.48% and 69.75% respectively at the highest concentration of 80 µg/ml. While other extracts EECC and MECC showed similar cancer cell antiproliferation pattern. All the extracts showed dose dependent antiproliferation activity while the control shows 100% proliferation. The IC50 values of each extract against the cancer cell line MDAMB231 are 35.89µg/ml, 82.12 µg/ml and 89.33 µg/ml respectively. According to Atjanasuppat *et al.,* 2009*,* a plant extract is regarded to have high antiproliferative active if their IC50 value is ≤ 20 µg/ml which is reagarded as an active form, IC50 > 20-100 µg/ml, which is denoted as moderately active, IC 50> 100- 1000 µg/ml is regarded as weakly active. However United States National Cancer Institute plant screening program stated that a crude extract will be generally considered to have cytotoxicty activity if its IC 50 value is < 30-40 µg/ml (Oskoueian *et al*., 2011). So adopting these criteria EaECC with IC 50 value of 35.89 µg/ml have shown strong cytotoxic activity against breast cancer cell lines MDAMD 231 while EECC and MECC showed moderate cytotoxic activity according to Atjanasuppat*et al.,* 2009.

Table: 26: Cytotoxic activity of *C.caesia* extracts in MDAMB 231 cell lines:

EaECC + Cells				EECC+ Cells			MECC+Cells		
Conc (µg/ml)	Absor-bance at 540nm	% proli feration	% inhi-bition	Absor-bance at 540nm	% proli feration	% inhi-bition	Absor-bance at 540nm	% proli feration	% inhi-bition
0.5	0.449	78.08	21.92	0.472	82.08	17.92	0.470	81.73	18.27
5	0.427	74.26	25.74	0.452	78.6	21.4	0.459	79.82	20.18
10	0.389	67.65	32.35	0.425	73.91	26.09	0.439	76.34	23.66
20	0.309	53.73	46.25	0.408	70.95	29.05	0.412	71.65	28.35
40	0.288	50.08	49.92	0.383	66.6	33.4	0.392	68.17	31.83
60	0.177	30.78	69.22	0.348	60.52	39.48	0.344	58.82	40.18
80	0.118	20.52	79.48	0.282	49.04	50.96	0.307	53.39	46.61
IC50 value	35.89µg/ml			82.12 µg/ml			89.33 µg/ml		
Control	0.575	100	0.00						

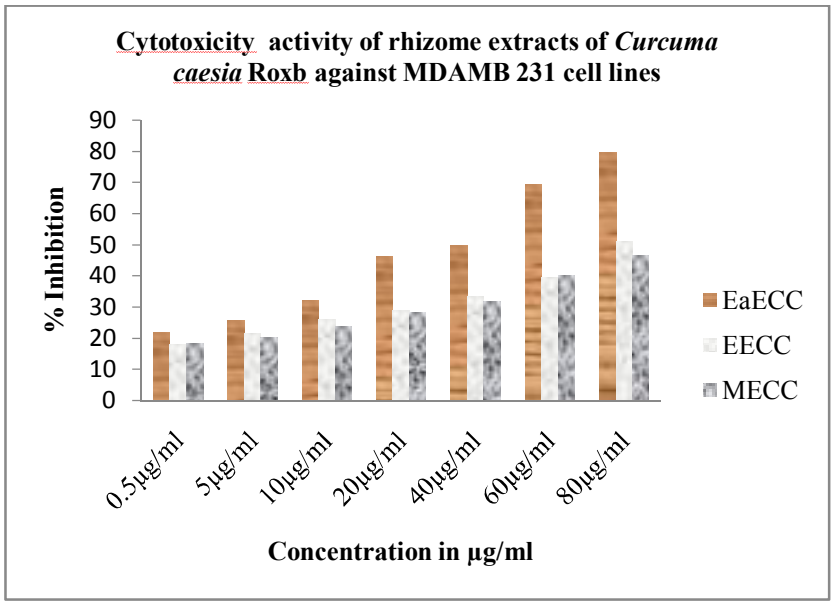


Fig: 44: Cytotoxic activity of *C.caesia* Roxb extracts against MDAMB 231 cell lines.

4.11.2: Cytotoxicity activity of the extracts against lung cancer cell lines Calu6:

The antiproliferating activitity of EaECC, EECC and MECC was also tested against lung cancer cell lines Calu6. Ethyl acetateextract demonstrates dose dependent antiproliferative activity against cancer cell lines Calu6 with the percentage inhibition of 32.37% at the lowest concentration, 5µg/ml and 69.75% at the highest concentration, 80µg/ml with the IC 50 value of 38.405µg/ml (table no. 27 and fig:45). EaECC showed strong cytotoxic activity while EECC and MECC showed moderate cytotoxic activity in cancer cell lines Calu6. EECC showed percentage inhibition of 33.18% at its lowest concentration, 5µg/ml and 48.87% at its highest concentration (80µg/ml) with the IC 50 value of 93.24 µg/ml. MECC showed moderate cytotoxic activity with the percentage inhibition of 31.81% at its lowest concentration (5µg/ml) and 50% at its highest concentration with the IC 50 value of 83.84 µg/ml. MECC showed more strong cytotoxic activity than EECC against Calu6 based on its low IC 50 value (table 24 & fig 31).

Table: 27: Cytotoxic activity of *C.ceasia* extracts inCalu 6 cell lines:

EaECC + Cells				EECC+ Cells			MECC+Cells		
Conc (µg/ml)	Absor-bance at 540nm	% proli ferati on	% inhi-bition	Absor-bance at 540nm	% proli feration	% inhi-bition	Absor-bance at 540nm	% proli feratio n	% inhi-bition
0.5	0.418	67.63	32.37	0.413	66.82	33.18	0.416	68.19	31.81
5	0.404	65.37	34.63	0.411	66.5	33.5	0.417	67.47	32.53
10	0.390	63.10	36.9	0.404	65.37	34.63	0.401	64.88	35.12
20	0.355	57.44	42.56	0.399	64.56	35.35	0.394	63.75	36.25
40	0.302	48.86	51.14	0.368	59.54	40.46	0.368	59.54	40.46
60	0.257	41.58	58.42	0.358	57.92	42.08	0.343	56.22	43.78
80	0.187	30.25	69.75	0.316	51.13	48.87	0.309	50.00	50
IC50 value	38.40µg/ml			93.24µg/ml			83.84µg/ml		
Contr ol	0.618	100	0.00						

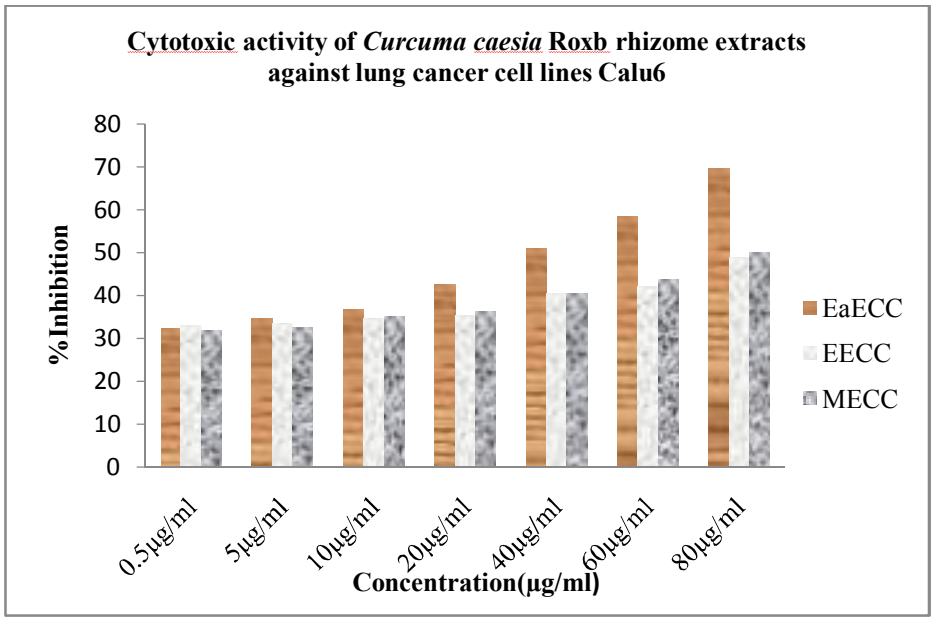


Fig: 45: Cytotoxic activity of *C.caesia* Roxb extracts against Calu6 cell line.

4.12: GC-MS analysis of the extracts:

4.12.1: GC-MS analysis of EaECC:

29 compounds were found to present in the GC-MS analysis of ethyl acetate extract of *Curcuma caesia* Roxb as shown in the following table no. 28. The compounds shown with respective peak values in the chromatograph were compared to the known compounds present in the NIST library.

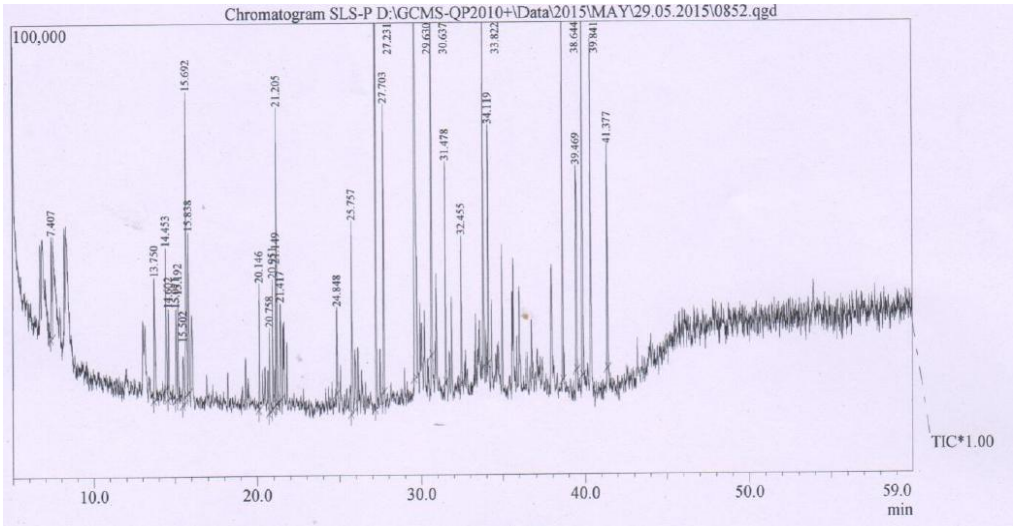


Fig: 46: GC-MS chromatogram of Ethyl acetate extract of *Curcuma caesia* Roxb.

4.12.2: GC-MS Analaysis of EECC:

Ethanolic extract of Curcuma caesia Roxb showed the presence of 44 compounds and all the compounds were match with the data in NIST library (table no. 29 and fig: 47).

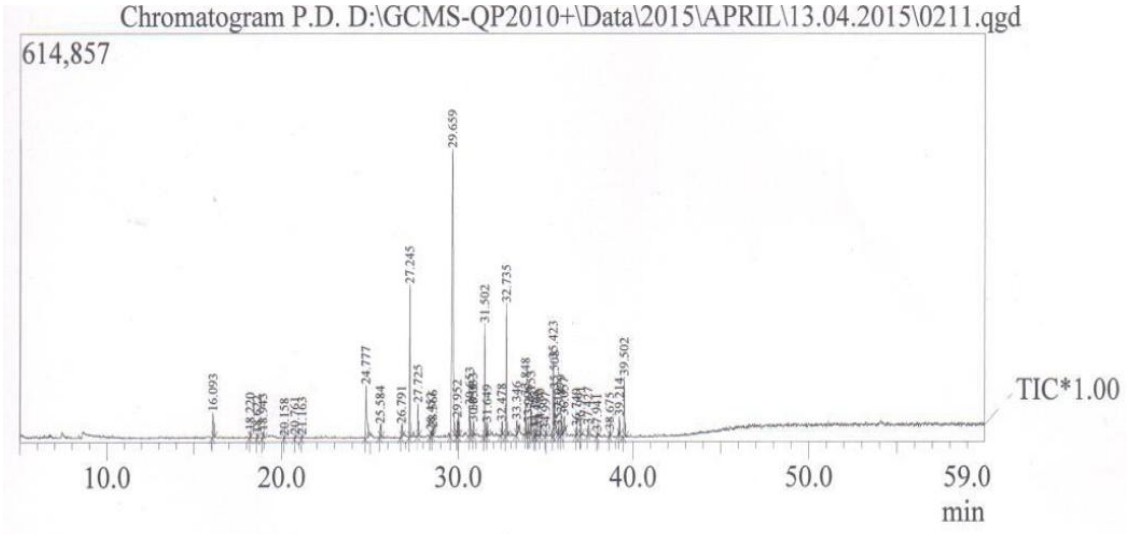


Fig: 47: GC-MS chromatogram of Ethanolic extract of *Curcuma caesia* Roxb.

Table: 28: GC-MS analysis of Ethyl acetate extract of *Curcuma caesia* Roxb:

Compound name	RT	%area	%height	Mol wt & Mol Formula
2(3H)-Naphthalenone,4,4a,5,6,7,8-hexahydro-	27.231	12.11	14.91	150,$C_{10}H_{14}O$
α-Gurjunene	29.630	13.23	7.74	204, $C_{15}H_{24}$
α-Limonene diepoxide	39.841	9.27	9.33	168, $C_{10}H_{16}O_2$
Cholesta-5,7,9(11)-trien-3-ol acetate	30.637	8.28	9.80	424,$C_{29}H_{44}O_2$
1H-Cyclopropa[3,4]benz[1,2-e]azulene-5,7b,9,9a-tetrol,1a,1b,4,4a,5,7a,8,9-octahydra-3(hydroxymethyl)-	33.822	6.02	7.38	476,$C_{26}H_{36}O_8$
Benzenesulfonyl chloride 2,4,6-tris(1-methylethyl)-	38.644	4.69	5.25	302,$C_{15}H_{32}ClO_2S$
Oxalic acid octyl propyl ester	15.692	4.84	3.80	244;$C_{13}H_{24}O_4$
3,3 dimethyl hexane	21.205	2.98	3.70	118,C_8H_{18}
3-ketois steviol	27.703	3.46	3.61	332,$C_{20}H_{28}O_4$
1H-2,8a-Methanocyclopenta(a)cyclopropa[e] cyclodecene-11-one, 1a,2,5,5a,6,9,10,10a,octahydro-	34.119	2.59	2.98	364,$C_{20}H_{28}O_6$
α-Limonene diepoxide	41.367	2.53	2.81	168, $C_{10}H_{16}O_2$
Estran-3-one, 17-(acetyloxy)-	31.478	2.07	2.77	332,C21H32O3

2-methyl-,(2 alpha, 5 alpha, 17-beta)				
Benzaldehyde, 3-(4-methoxyphenoxy	39.469	3.73	2.56	228,$C_{14}H_{12}O_3$
3,3 -dimethylhexane	25.757	2.54	2.41	114,C8H18
Oxalic acid octyl propyl ester	15.838	2.06	2.02	244, $C_{13}H_{24}O_4$
Preg-4-ene-3-one,17-alpha hydroxyl-17-beta-cyano	32.455	1.60	1.85	313,C_7H_{16}
1-Iodo-2-methylnonane	14.453	2.34	1.77	268,$C_{10}H_{21}I$
Cineole	13.750	2.10	1.54	154,$C_{10}H_{18}O$
Citrnellol,dihydro	20.951	1.30	1.63	158, $C_{10}H_{22}O$
n-Nonyl Iodide	20.146	1.27	1.58	254,$C_9H_{19}I$
1-Heptanol-6-methyl	21.147	1.25	1.76	130,$C_8H_{18}O$
Sulfurous acid decyl 2-propylester	24.848	0.92	1.08	264,$C_{13}H_{28}O_3S$
Isooctanol	15.192	1.38	1.28	130,$C_8H_{18}O$
Acetic acid trifluoro-,3-methylbutylester	15.071	1.38	1.14	184,$C_7H_{11}F_3O_2$
Shellsol	14.602	1.26	1.13	128,C_9H_{20}
3,4-dimethylheptane	7.407	1.53	1.28	128,C_9H_{20}
Oxalic acid allyl pentadecyl ester	20.758	0.89	1.06	340,$C_{20}H_{36}O_4$
1,6-Heptadien-4-ol	15.502	0.91	0.62	112,$C_7H_{12}O$

Table: 29: GC-MS analysis of Ethanolic extract of *Curcuma caesia* Roxb.

Sl No	Compounds Name	Retention time	Molecular weight& molecular formula
1	α Gurjunene	29.659	204; $C_{15}H_{24}$
2	2(3H)-Naphthalenone,4,4a,5,6,7,8-hexahydro-	27.245	150,$C_{10}H_{14}O$
3	Hexadecanoic acid methyl ester	32.735	270; $C_{17}H_{34}O_2$
4.	Viridiflorol	31.502	222;C15H26O
5	9,12 octadecanoic acid methyl ester (E, E)	35.423	294; $C_{19}H_{34}O_2$
6	4,5,8Trimethylpsorale (Furanocoumarin)	39.502	228;$C_{14}H_{12}O_3$
7	9 hexadecanoic acid methyl ester (Z)-	35.505	268 ;$C_{17}H_{32}O_2$
8	Andrographolide	27.725	350; $C_{20}H_{30}O_5$
9	All trans retinoic acid (ATRA)	33.848	300;$C_{20}H_{28}O_2$
10	2 Furanmethanol	16.093	98; $C_5H_6O_2$
11	2,3-dimethylfumaric acid	24.77	144, $C_6H_8O_4$
12	4-pregnen-6-alpha, 17-alpha-diol-3, 20-dione	30.653	346, $C_{21}H_{30}O_4$
13	Cyclopentanemethanol	29.952	100, $C_6H_{12}O$
14	Benzenemethanol, alpha-methyl-alpha-2,5,7-octatrienyl	25.584	228,$C_{16}H_{20}O$
15	1,1'-Carbonylimidazole	26.791	162,$C_7H_6N_4O$

16	3-tetradecane (Z)	39.214	196, C14H28
17	Cyclohexanol,1-acetyl-2-ethylidene	37.427	168, $C_{10}H_{16}O_2$
18	Cyclohexanol, 2-methyl-5-(1-methylethenyl)-,acetate,(1 alpha, 2 beta, 5 alpha)	32.478	196, $C_{12}H_{20}O_2$
19	1H-3a, 6-Methanoazulene-3-carboxylic acid, octahydro-7,7-dimethyl-8-methylene,[3S-(3 alpha, 3a-alpha, 6alpha, 8a alpha0]	31.699	234,$C_{15}H_{22}O_2$
20	Cyclododeca-5,9-dien-1-ol,2-methyl-,(Z,Z)-	31.509	194, $C_{13}H_{22}O$
21	3,4-Hexandiol,2,5-dimethyl	33.346	146,$C_8H_{18}O_2$
22	Oxalic acid butyl propyl ester	18.217	188, $C_9H_{16}O_4$
23	Anthiaergostan-5,7,9,13-tetraen, 15-acetoxy	35.717	436,$C_{30}H_{44}O_2$
24	Alanine, N-acetyl-3-phenyl-N-(trifluoroacetyl)-,methyl ester,L	36.059	317,$C_{14}H_{14}F_3NO_4$
25	Propane, 2-Nitro	20.158	89, $C_3H_7NO_2$
26	Ethanone, 1-[6-(4-methyl-1,2,5-oxadiazol-3-yl)-4H-pyrazolo[3,4-c]-1,2,5-oxadiazol-4-yl]-	28.453	234, $C_8H_6N_6O_3$
27	Tartrononitrile, methyl-, acetate (ester)	28.569	138,$C_6H_6N_2O_2$
28	Benzoic acid, 4-benzyloxy-3[1-methoxyethoxy]-butan-2-yl ester	30.858	358,$C_{21}H_{26}O_5$

29	3-Hydroxy-12-ketobisnocholanic acid	34.002	362,$C_{22}H_{34}O_4$
30	1H-Cyclopropa[a]naphthalene, 1a,2,3,5,6,7, 7a, 7b-octahydro-1,1, 7, 7a tetramethyl-,[1aR-(1a.alpha, 7 alpha, 7a alpha, 7b alpha)]-	34.153	204,$C_{15}H_{24}$
31	Methacrylic anhydride	18.624	154,$C_8H_{10}O_3$
33	Isopropylbenzylketone	18.945	162, $C_{11}H_{14}O$
34	Dibenzene,1,1', 4,4'-bis(1,2-ethanediyl)-2-[hydroxyl(4-methylphenyl)methyl]-3-isopropoxy-	38.676	386,$C_{27}H_{30}O_2$
35	Bicyclo[2.2.1]heptan-2-ol, 1-methyl-,acetate	36.987	168, $C_{10}H_{16}O_2$

4.13: UV-Visible Spectrum of EaECC ans EECC:

4.13.1: UV-visible spectrum of Ethyl acetate extract of *Curcuma caesia* Roxb:

The qualitative UV-Visible spectrum profile of ethyl acetate extract of the rhizome of *Curcuma caesia* Roxb was selected at wavelength from 260 to 400nm due to sharpness of the peaks in between this region. The profile showed the peaks at 300nm, 320 and 380nm with the absorption of 1.196, 0.863 and 0.371 respectively (table 30; figure 48).

Table: 30: UV Visible peak values of ethyl acetate extract of *Curcuma caesia* Roxb:

Wavelength (nm)	Absorption	Wavelength (nm)	Absorption
200	0.119	380	0.371
220	0.163	400	0.276
240	0.091	420	0.175
260	0.114	440	0.122
280	0.153	460	0.091
300	1.196	480	0.072
320	0.863	500	0.055
340	0.146	520	0.047
360	0.220	540	0.00

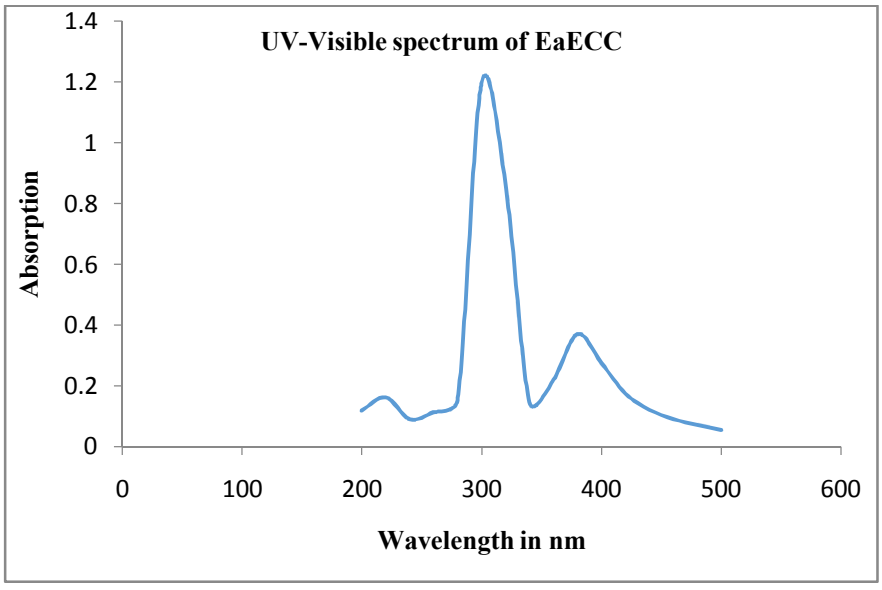


Fig: 48: UV-Visible spectrum of Ethyl acetate extract of *Curcuma caesia* Roxb.

4.13. UV-Visible peak values of Ethanolic extract of *Curcuma caesia* Roxb:

The UV-Visible profile of ethanolic extract of *Curcuma caesia* Roxb was selected at the wavelength of 200 to 400nm and the spectrum profile showed the peaks at 200, 300, 320, 340 and 360nm with the absorption of 0.748, 1.067, 0.737, 0.494 and 0.294 respectively (table 31 and figure 49).

Table: 31: UV-Visible peak values of Ethanolic extract of *Curcuma caesia* Roxb:

Wavelength (nm)	Absorption	Wavelength (nm)	Absorption
200	0.748	340	0.494
220	0.00	360	0.294
240	0.00	380	0.180
260	0.00	400	0.107
280	0.00	420	0.074
300	1.067	440	0.021
320	0.737	460	-0.025

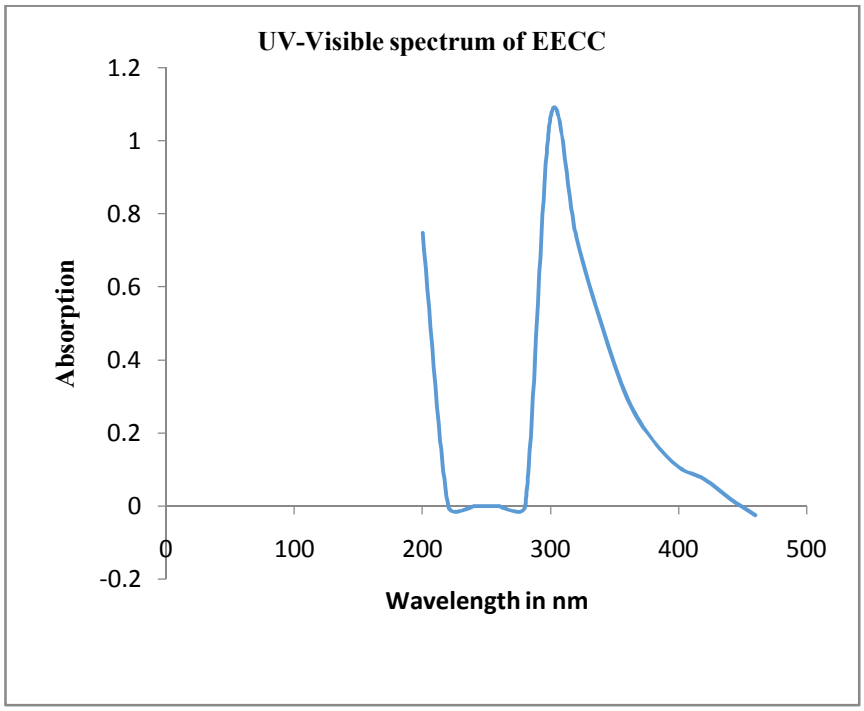


Fig: 49: UV-Visible Spectrum of Ethanolic extract of *Cucrcuma caesia* Roxb.