Chapter 4
RESULTS

4.1 Yield of Plant Material and Extraction

The yield was found to be 5.8%.

4.2 *In-vitro* analysis

4.2.1 Preliminary Phytochemical Screening

The table 4.1 indicates the presence of carbohydrates, reducing sugars, tannins, saponins, flavonoid, steroids, alkaloids, anthraquinones, and glycosides.

Table 4.1: Preliminary phytochemical screening of *Bambusa tulda* leaf extract

Chemical compounds	Results
Saponins	+
Steroids	+
Alkaloids	+
Tannins	+
Carbohydrates	+
Flavonoid	+
Anthraquinone	+
Glycosides	+
Reducing sugars	+

- = Compound not detected; + = Compound detected

4.2.2 Determination of Total Phenolic Content

The biochemical constituent such as total phenolic, content in aqueous methanolic extract of *Bambusa tulda* were found to be 17.494±0.01mg GAE /g.

4.2.3 Determination of Total Flavonoids and Flavonol content

The total flavonoid and flavonol of BT was 176.35±0.03 mg quercetin equivalent (QE)/g and 96.2±0.01 mg QE /g of extract as calculated with standard curve.

4.2.4 Determination of Total Proanthocyanidins

The total proanthocyanidin of BT was found to be 11±0.86µg catechin equivalent (CE)/g of extract as

calculated with standard curve.

4.2.5 Assessment of antioxidant activity

4.2.5.1 Free Radical scavenging assay-2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) method

DPPH radical scavenging activity in the BT was found to be decreasing as the concentration decreases.

DPPH scavenging activity is found highest at the concentration 200mg/ml. There is significant activity noticed when compared with standard ascorbic acid. The activity was found 20 to 78% as increase in concentration respectively. Previous studies indicated the methanolic extract is more suitable solvent for the extract preparation since it also inhibit the degradation of polyphenols present in the plants by neutralizing the activity of polyphenol oxidase (Goyal et al., 2010). Hu and his co-workers discussed the antioxidant activity of bamboo. In their study they have shown bamboo exhibits a good concentration dependent scavenging activity of the $DPPH^{\cdot}$ radical activity. Their study indicated bamboo as an effective

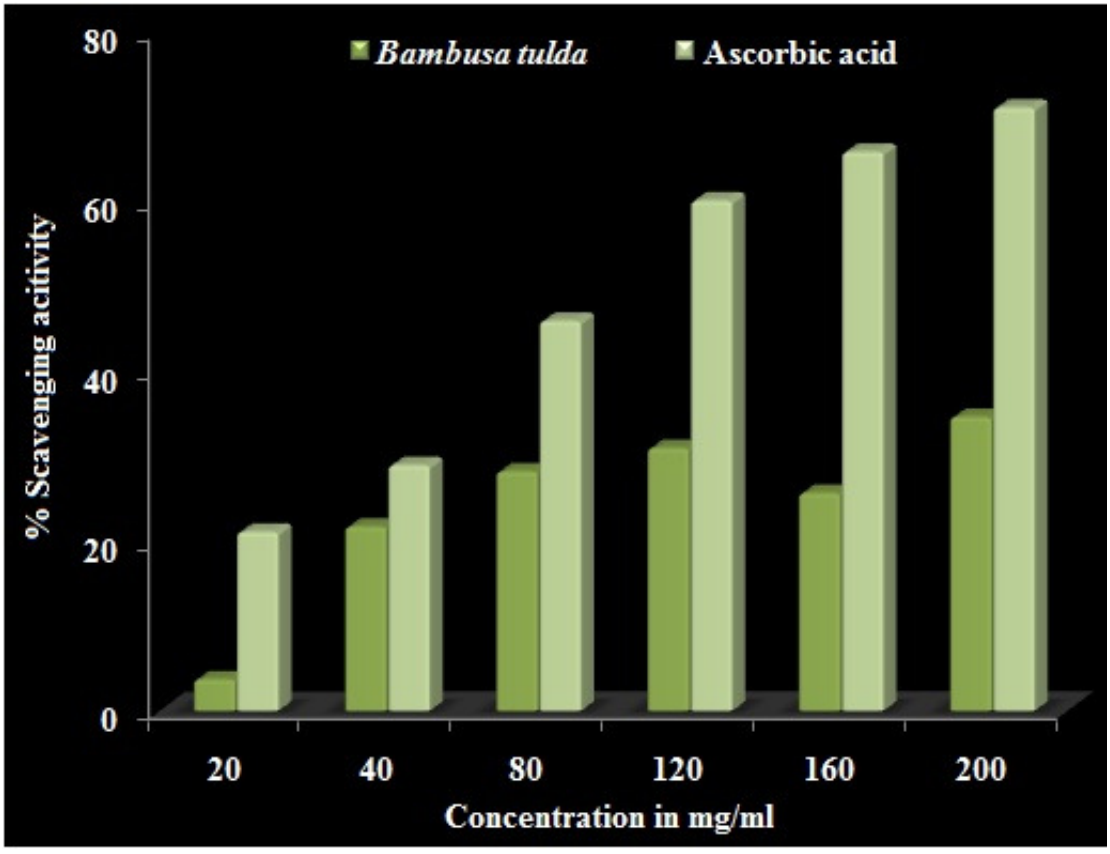


Figure 4.1: DPPH scavenging activity of *B. tulda* leaf extract compared to standard ascorbic acid

natural antioxidant.

4.2.5.2 Ferric reducing power assay (FRP)

The result indicated the increase in optical density (activity) with regards to increase in concentration. The result show significant increase when compare to standard BHT.

Figure indicated the reducing power was found to be proportional to the BT concentration and was increasing steadily with increase in concentration. The theory behind the reductive capability is mainly transformation of Fe^{3+} to Fe^{2+} in presence of the crude extract and the BHT used as standard.

It is also noted that the absorbance of BT and the BHT coinciding at 60µg/ml concentration

- At 100 µg/ml the absorbance (OD) of BT and BHT was found to be almost two fold i.e. 0.16 and 0.08 respectively. So it can be said that BT has the potential to scavenge more radical than BHT. Though there are more substantial proof might be needed to claim the said statement.

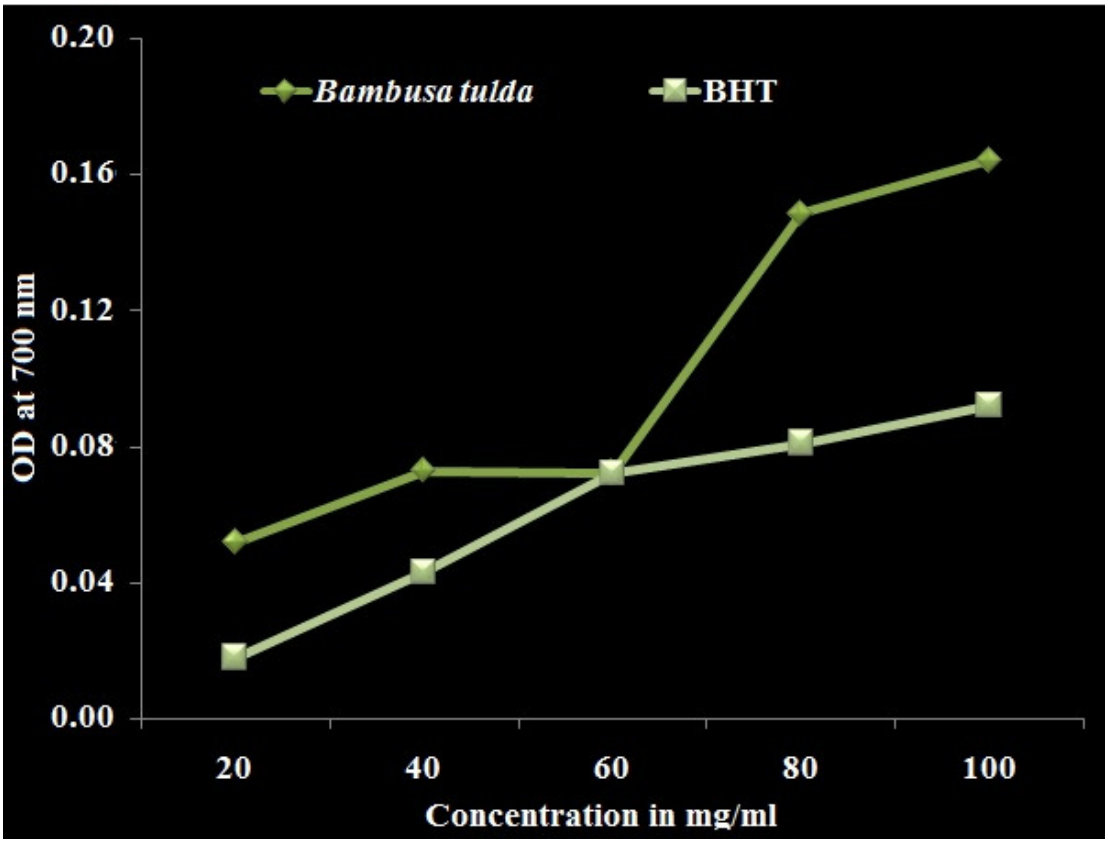


Figure 4.2: Ferric reducing power assay of *B. tulda* leaf extract compared to standard BHT

4.2.5.3 Hydrogen peroxide scavenging activity

The H_2O_2 scavenging activity of different concentration of leaf extracts compound to standard ascorbic acid is shown in the figure 4.3. Increase in concentration is directly reciprocates the increase in % scavenging activity. At 200 mg/ml BT has shown the similar pattern of % scavenging activity as the standard ascorbic acid.

4.3 Correlation studies

The resultant antioxidant and free-radical scavenging activity of BT are inter-correlated using correlation coefficient method and represented in Table 4.2 and figure 4.4 shown below. TP has shown positive correlation with TF(R^2=0.521) and was positively co-related with other free radical scavenging parameter such as DPPH (R^2=0.791), FRP (R^2=0.697) and

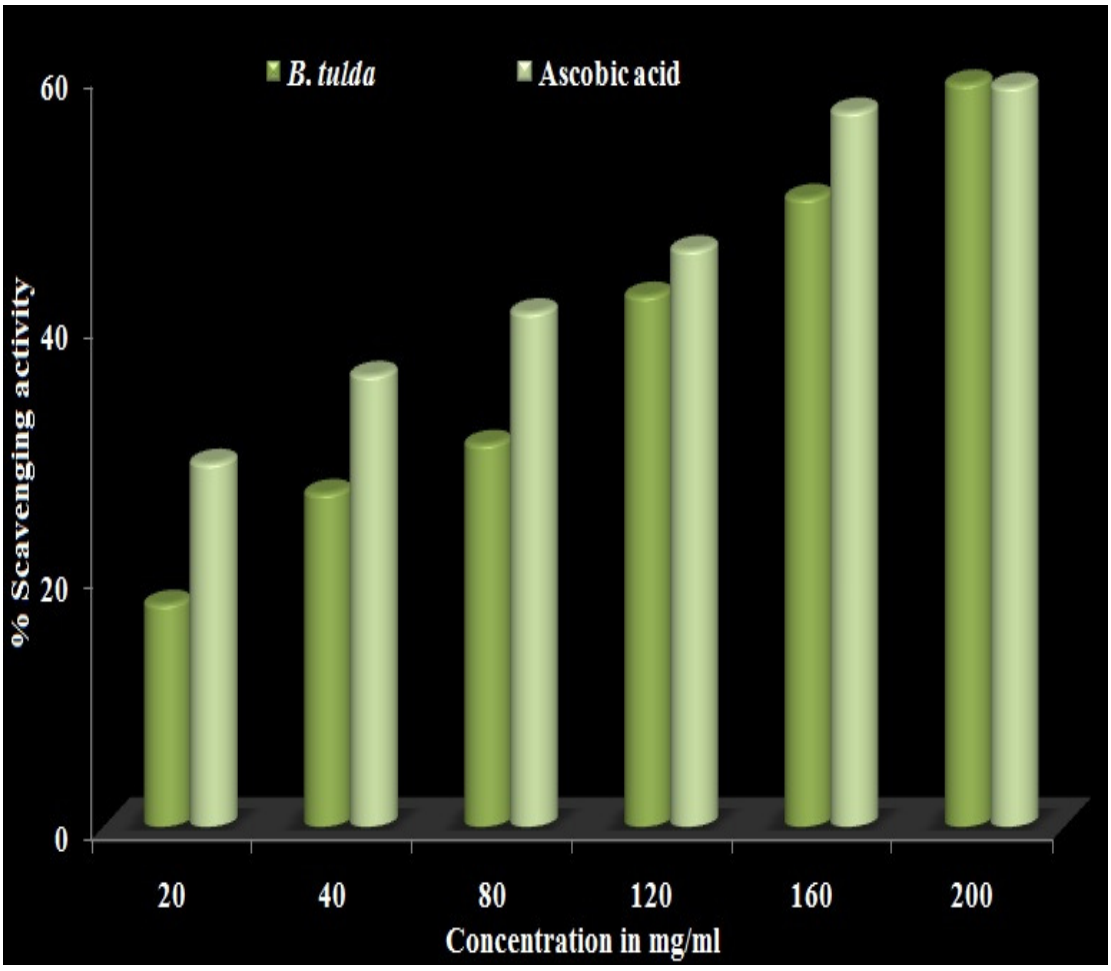


Figure 4.3: Hydrogen peroxide scavenging activity of *B. tulda* leaf extract compared to standard ascorbic acid

Table 4.2: Correlation between different antioxidant parameters of *Bambusa tulda* leaf

	TP	TF	DPPH	H_2O_2	FRP
TP	**1**				
TF	**0.521**	**1**			
DPPH	**0.791**	**0.272**	**1**		
H_2O_2	**0.692**	**0.793**	**0.369**	**1**	
FRP	**0.882**	**0.249**	**0.371**	**0.489**	**1**

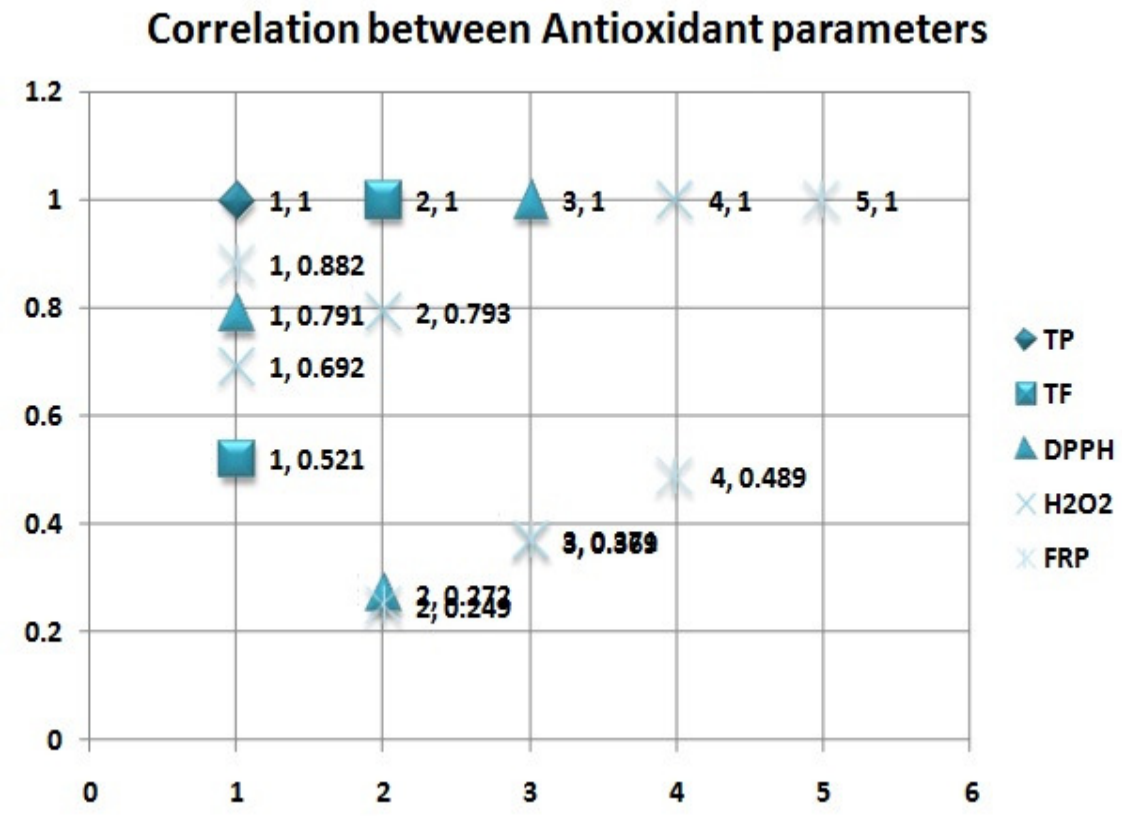


Figure 4.4: Correlation between different antioxidant parameters of *Bambusa tulda* leaf

H2O2 (R^2=0.882). TF was also seen positively correlated with hydrogen peroxide scavenging (R^2=0.792), DPPH (R^2=0.272) and FRP (R^2=0.242). Other parameter was also noted positively correlated such as DPPH v/s H2O2 (R^2=0.362) and DPPH v/s FRP (R^2=0.371).

4.4 GC-MS analysis

The hydromethnolic crude extract of *Bambusa tulda* leaf, analyzed by GC-MS had led to the identification of seven different organic compounds (Figure 4.5 a-e). The identified compounds with information on retention time, peak height and peak area are listed in table 4.3.

4.5 *In vivo* studies

4.5.1 Acute Toxicity Test

LD_{50} of BT extract was 988.13mg/kg body weight in mice**.**

4.5.2 Induction of Hyperglycemia

Prior to the experiment, rats were divided in six groups. Except group one, all other groups were administered with the alloxan. The blood glucose of the rats was checked before starting the experiment and it was not significantly different. There was a significant increase in glucose level was seen after 3days alloxan administration and was considered as the Diabetic group.

4.5.3 Effect of BT on body weight

The animals treated with BT has shown an increase in body weight. Maximum increase was seen in standard drug than the animals treated with extract (Figure 4.6).

4.5.4 Effect of BT on glucose level

A significant anti-hyperglycemic activity was noticed from the first week onwards. The decrease was observed prominent up to 6th week in experimental animals receiving the high dose of BT. The significant (p<0.05) increase in blood glucose level was seen after alloxan administration when compared to normal control rats. The BT administration significantly (p<0.001) reduced the blood glucose levels in diabetic rats as compared with diabetic control rats. Even, the higher dosage of BT was seen more effective than the lower dosage of BT. (Figure 4.7)

4.5.5 Enzyme Assays

4.5.5.1/2. Glutathione peroxidase (GSH-Px, EC 1.11.1.9) and *Super-oxide dismutase (SOD, EC 1.15.1.1)*

The SOD and GPx concentration in liver of the normal and experimental rats were studied (Figure 4.8 and 4.9).

Table 4.3: Phytochemical constituents of *Bambusa tulda* leaf using GC-MS analysis

Sl. No	IUPAC Name	Chemical Formula	Common Name	RT (min)	Height	Area	Peak Area%
1	4-Hydroxybenzoic acid	$C_7H_6O_3$	p-Hydroxy benzoic acid	2.25	1219	212.95	37.53
2	2-Hydroxybenzoic acid	$C_7H_6O_3$	Salicylic acid	6.27	828	354.41	62.47
3	(2E)-3-(2-hydroxyphenyl) prop-2-enoic acid	$C_9H_8O_3$	o-Coumaric acid	5.51	1315	150.48	8.31
4	(2E)-3-(4-hydroxyphenyl) prop-2-enoic acid	$C_9H_8O_3$	p-Coumaric acid	5.22	9985	1660.15	91.69
5	2,4-Dihydroxybenzoic acid	$C_7H_6O_4$	β-Resorcylic acid	4.81	165	25.25	100
6	4-Hydroxy-3-methoxybenzoic acid	$C_8H_8O_4$	Vanillic acid	3.32	115	16.63	100
7	(2E)-3-(4-hydroxy-3-methoxy-phenyl)prop-2-enoic acid	$C_{10}H_{10}O_4$	Ferulic acid	5.78	329	80.55	100

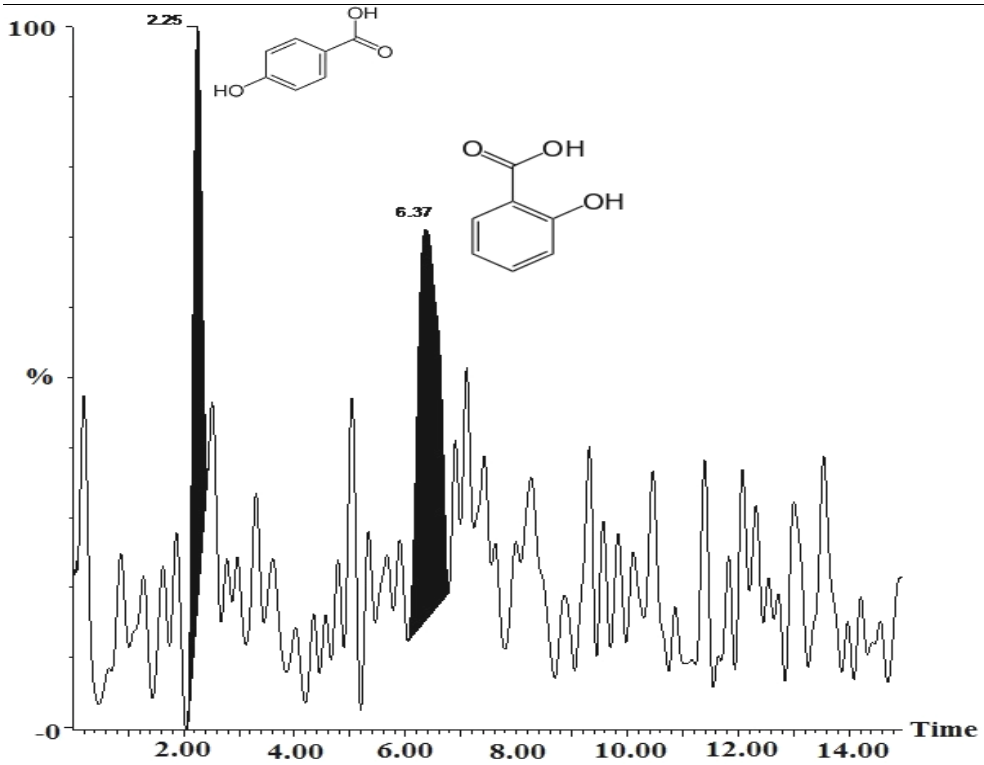


Figure 4.5a: GC–MS of hydromethanolic fraction of *Bambusa tulda* leaf showing the presence of P- hydroxy benzoic acid and Salicylic acid

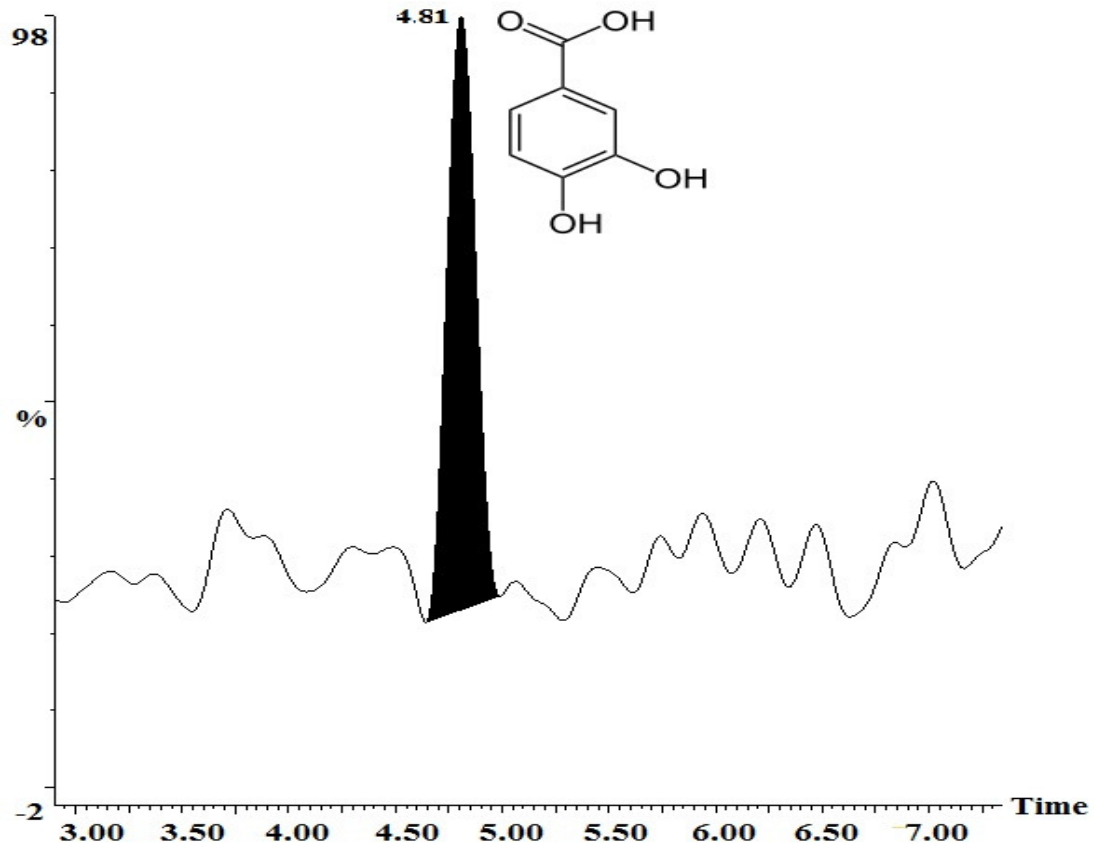


Figure 4.5b: GC–MS of hydromethanolic fraction of *Bambusa tulda* leaf showing the presence of 2,4-dihydroxy benzoic acid

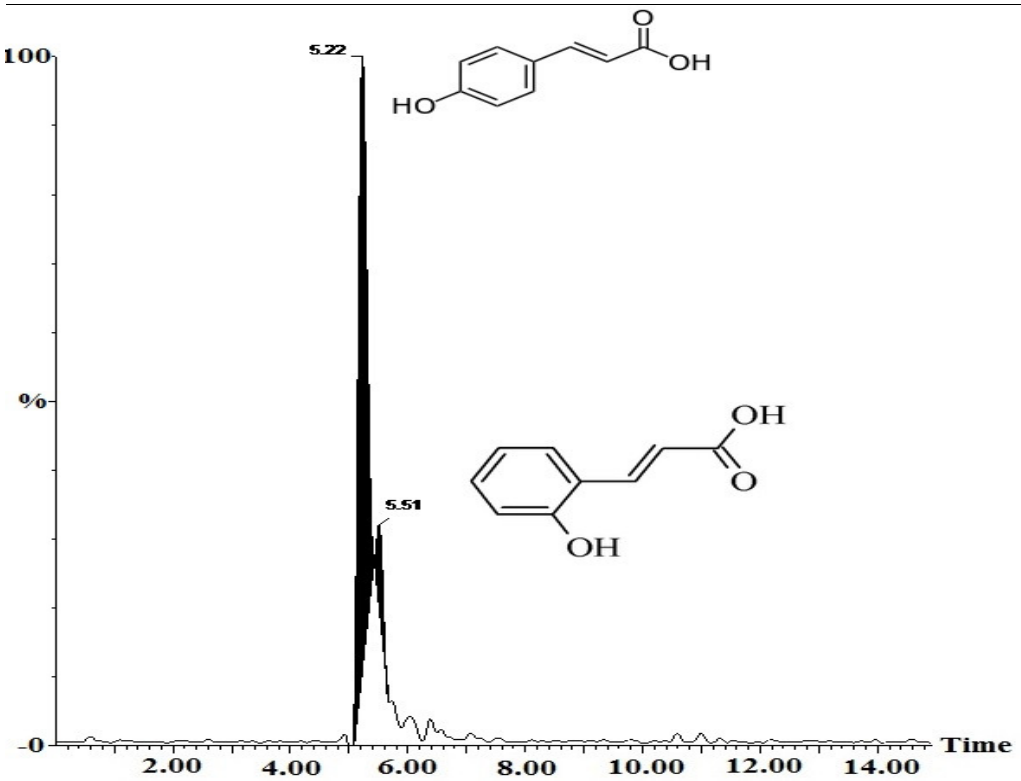


Figure 4.5c: GC–MS of hydromethanolic fraction of *Bambusa tulda* leaf showing the presence of p-coumaric acid and o-coumaric acid

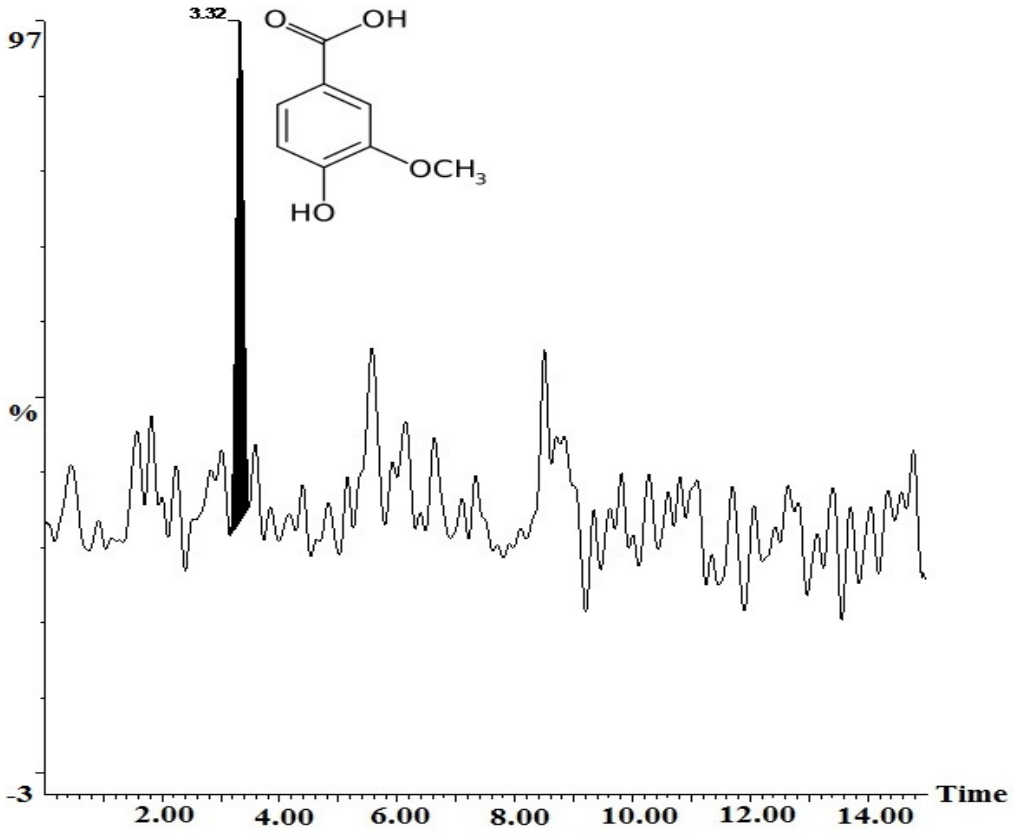


Figure 4.5d: GC–MS of hydromethanolic fraction of *Bambusa tulda* leaf showing the presence of vanillic acid

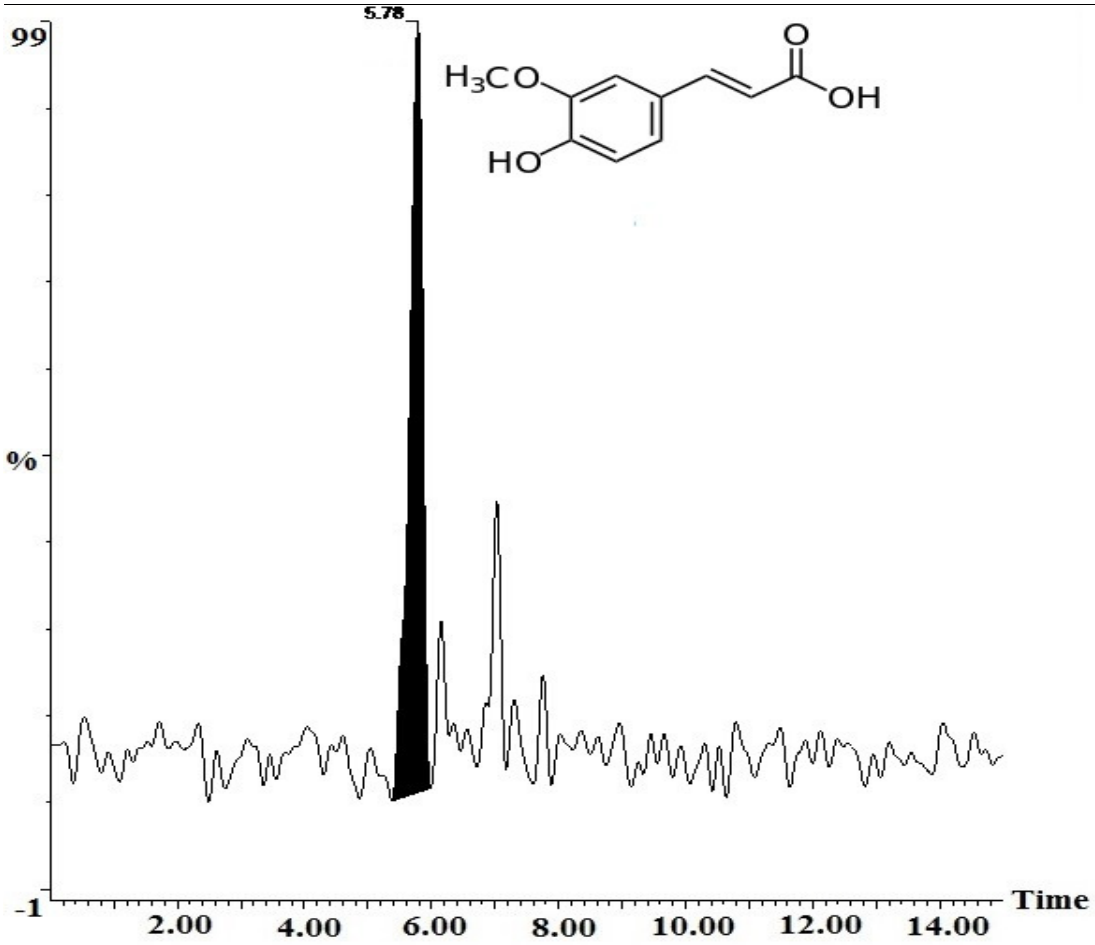


Figure 4.5e: GC–MS of hydromethanolic fraction of *Bambusa tulda* leaf showing the presence of ferulic acid

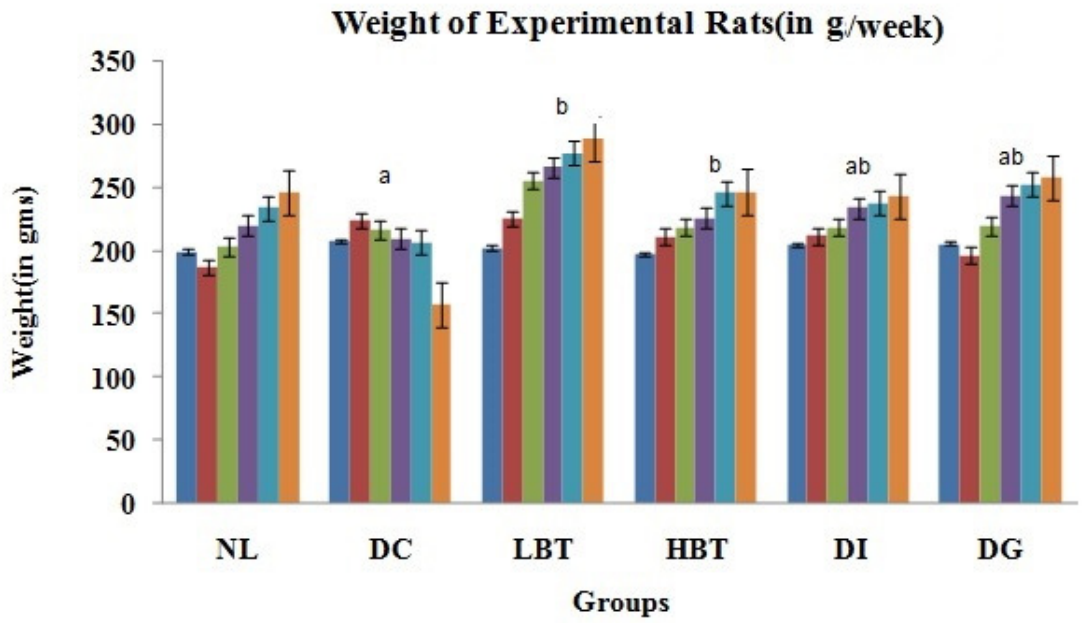


Figure 4.6:Effect of hydromethanolic extract of *Bambusa tulda* leaf on body weight in different experimental groups. Those are not sharing the common letters (a & b) are significantly different.

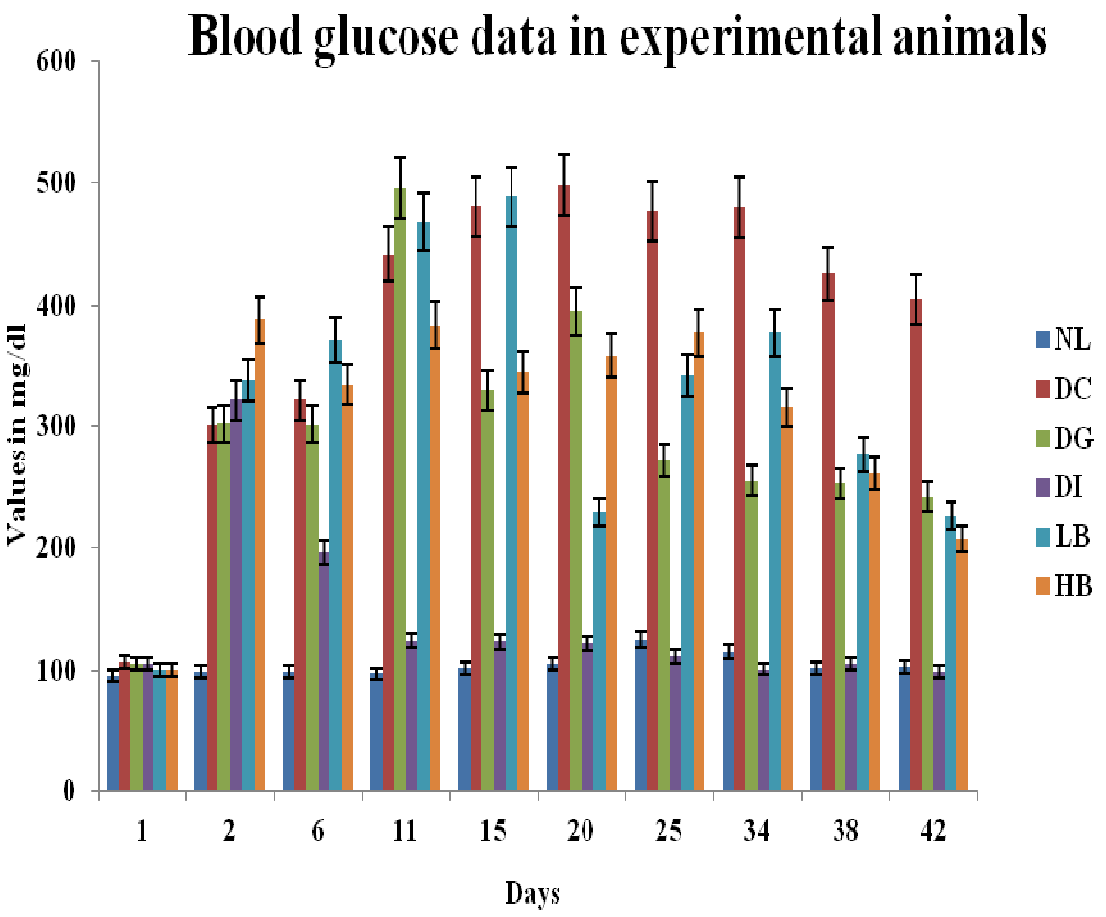


Figure 4.7: Effect of *B. tulda* leaf extract on fasting blood glucose NL, Normal; DC, Diabetic Control; DG, Diabetic Glibenclamide; DI, Diabetic Insulin; LB, Low dosage of BT (100 mg/kg b.w.); HB, High dosage of BT (200 mg/kg b.w.). Values are mean ± SE of nine rats per group (n=9). Statistical analysis was done by one-way ANOVA between groups and values were considered significant at $p<0.05$.

A significant reduction was seen in these antioxidant enzymes in the kidney of diabetic control group. A lower and higher dosage of BT had increase the antioxidant level of SOD (24.81%), GPx (31.60%) in liver. Higher dose of BT indicated a significant effect. Though the result was not near to normal. Then also BT extract was able to maintain the antioxidant level.

4.5.5.3 Lipid Peroxidation (LPO)

A significant decrease of malondialdehyde (MDA) in liver with the higher dose of kidney was recorded when compared with the diabetic control group (Figure 4.10). BT administration, insulin and

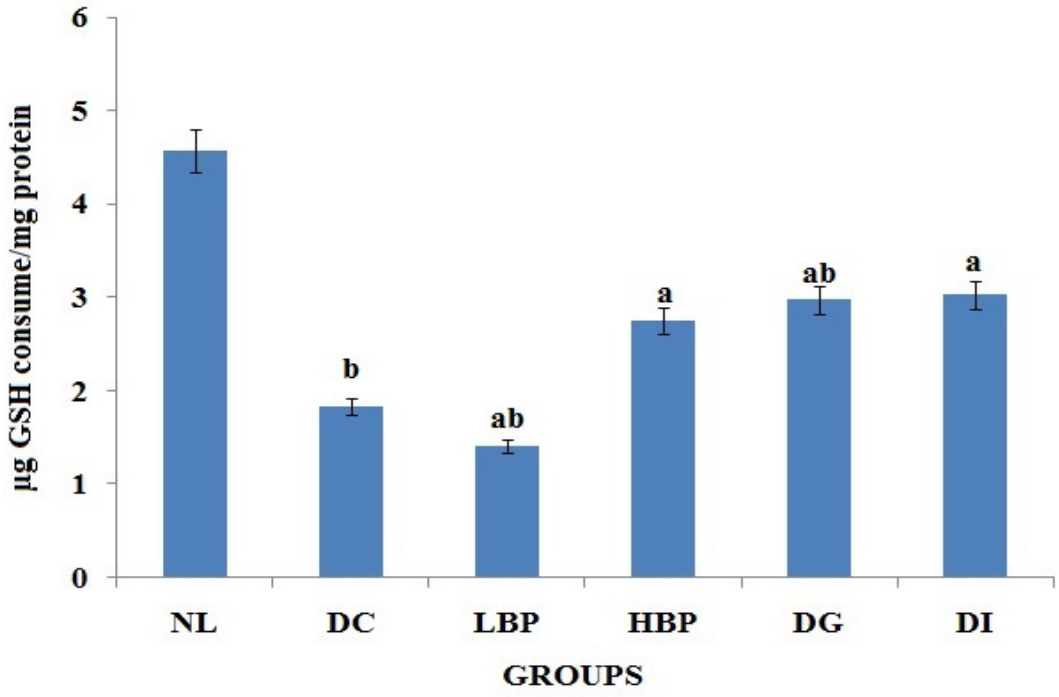


Figure 4.8: Effect of *B. tulda* leaf extract supplementation on glutathione peroxidase in liver of experimental rats. Values are means + S.E. (n=6 animals/groups) in liver of experimental animals. Results were considered significant between the groups at $p < 0.001$ in a comparative studies with DC. Those are not sharing the common letters (a & b) are significantly different.

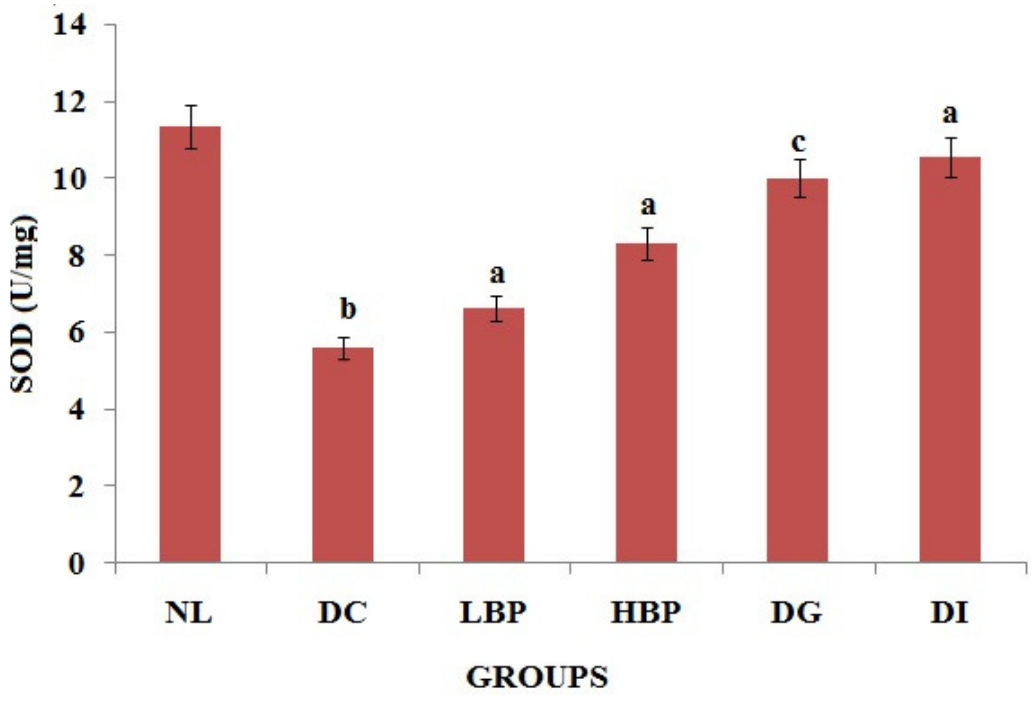


Figure 4.9: Effect of *B. tulda* leaf extract supplementation on superoxide dismutase in liver of experimental rats. Values are means + S.E. (n=6 animals/groups) in liver of experimental animals. Results were considered significant between the groups at $p < 0.001$ in a comparative studies with DC. Those are not sharing the common letters (a, b & c) are significantly different.

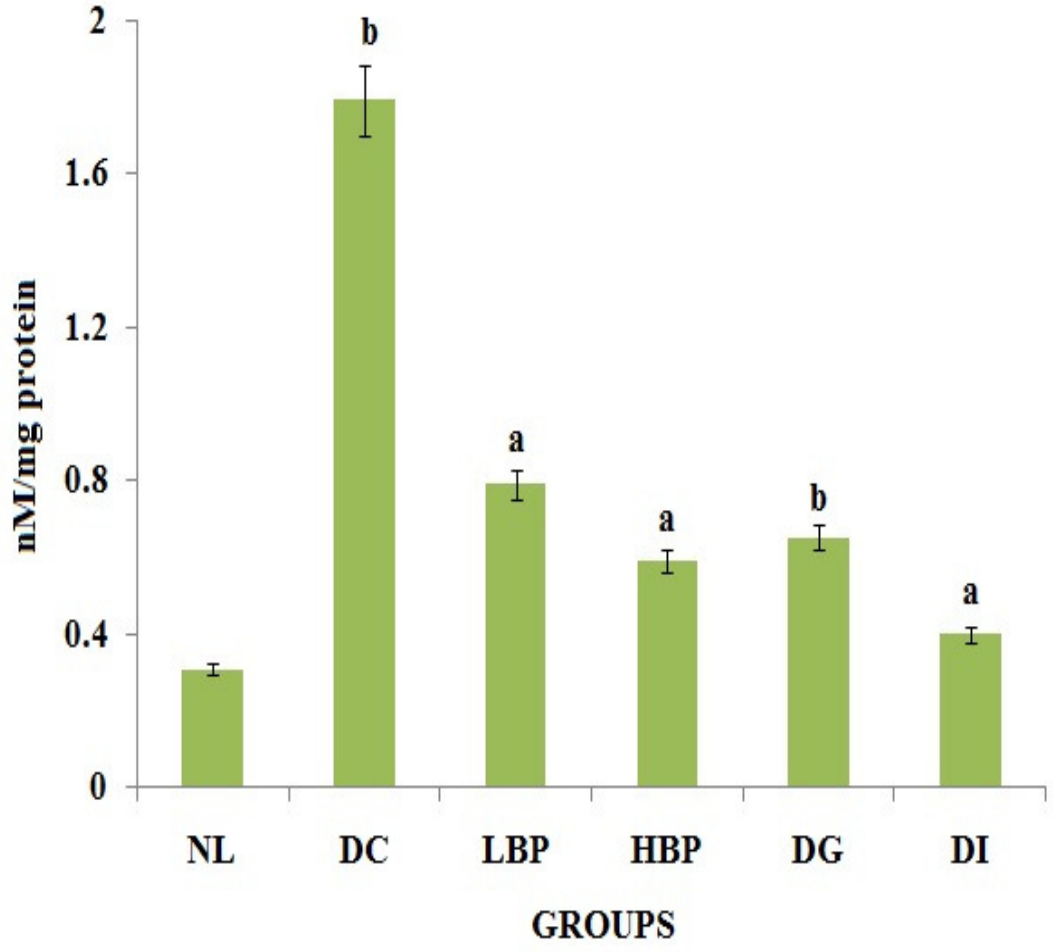
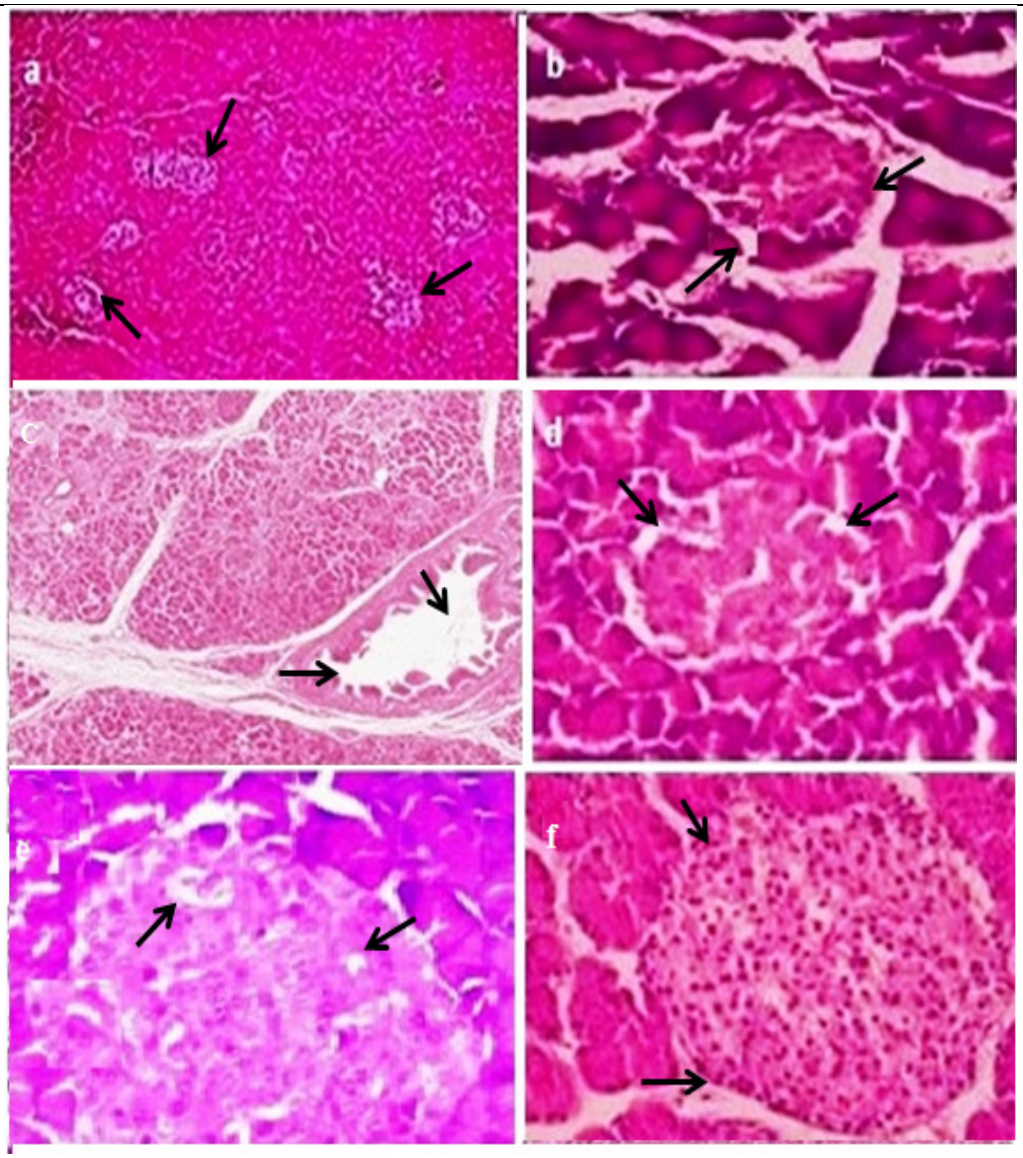


Figure 4.10: Effect of *B. tulda* leaf extract supplementation on lipid peroxidation in liver of experimental rats. Values are means + S.E. (n=6 animals/groups) in liver of experimental animals. Results were considered significant between the groups at $p < 0.001$ in a comparative studies with DC. Those are not sharing the common letters (a & b) are significantly different.

glibenclamide tends to bring down the MDA level to close to normal. Higher dose has shown better result than the lower dosage. It's was more effective than glibenclamide.

4.6 Histopathological studies

Reductions in number of Islets were observed in the pancreas of alloxan induced diabetic animals (Figure 4.11). The rats treated with lower dose of BT (100 mg/kg body weight) restored a much lesser number of β-cell region than (Figure 4.11C) the higher dose of BT (200 mg/kg body weight) (Figure 4.11D). The rejuvenation of β-cells was analogous to glibenclamide (Figure 4.11E) and insulin treated groups (Figure 4.11F).



Bambusa tulda extract effect on pancreas histopathology in normal and alloxan induced diabetic rats. (a) Normal control: Normal control group display the normal islet (arrow) (b) Diabetic control: Diabetic control rat histopathology display the islet enlargement and necrosis (arrow) (c)LBT (100 mg/kg BW): BT tested drug histopathology display increasing islets and necrosis (arrow) (d) HBT (200 mg/kg BW) (e) Glibenclamide (400 µg/kg BW): Glibenclamide treated group rat histopathology showing normal islet close to normal control group (f) Insulin (1U/ kg BW): Insulin treated group rat histopathology showing normal islet close to normal control animal group. For each group 6 animals were examined and 30 slides were captured. (Original magnification, 40X)