Results and Discussion
"There are a great many indigenous drugs of extreme utility but little known to students of western medicine. In numerous cases where allopathic treatment fails, indigenous system of medication succeeds".

Nadkarni

Hyperlipidemia is defined as a state of elevated cholesterol, triacylglycerol, phospholipids and free fatty acids either singly or in any combination. Utilizing epidemiological methods, its presence has been sought in cohorts screened for risk factors important in recognizing those at hazard for premature Coronary heart disease (Raonskov, 2000).

Available evidence indicating a possibility to inhibit the development of atherosclerosis and to reduce the mortality rate due to associated diseases in humans, forms the rationale behind prevention of cardiovascular disease. It consists of simultaneous modification of all concomitant risk factors such as LDL cholesterol and triacylglycerol levels with a number of clinical benefits (Fruchart, et al., 1998).

The experimental set of animals were fed on diet supplemented with cholesterol and groundnut oil to produce hyperlipidemia for a specified duration of 30 days and were diagnosed as hypercholesterolemic, hyperlipidemic and atherosclerotic, hence these words are used as synonyms in this presentation.

The Electrocardiogram (ECG) is the most useful tool for arrhythmia detection, and if a 12 lead recording can be obtained during symptoms, it is
PLATE I

a. Aorta of normal rat showing normal architecture. H & E 120x

b. Aorta of *Eclipta alba* treated normal rats showing normal architecture. H & E 120x

c. Aorta of Hypercholesterolemia induced rat showing intimal encrustation. H & E 600x

d. Aorta of Hypercholesterolemia induced rat showing fatty vacuolation in the intima. H & E 600x

e. Aorta of High fat diet + *Eclipta alba* treated rat showing almost normal architecture. H & E 240x

f. Aorta of High fat diet + Clofibrate treated rat showing almost normal architecture. H & E 120x
PLATE II

a. Liver of control rat showing normal architecture. H & E 100x

b. Liver of *Eclipta alba* treated normal rat showing normal architecture. H & E 100x

c. Liver of Hypercholesterolemia induced rat showing inflammatory infiltrate around portal triad (I) & dilated sinusoids (DS). H & E 40x

d. Liver of Hypercholesterolemia induced rat showing fatty vacuolation of parenchymal cells. H & E 100x

e. Liver of High fat diet + clofibrate treated rat showing less congestion & sinusoidal dilation. H & E 100x

f. Liver of High fat diet + *Eclipta alba* treated rat showing normal architecture. H & E 100x
a. Heart of normal rat showing normal architecture i.e C/S endocardium (E) and myocardium (M). H & E 20x

b. Heart of *Eclipta alba* treated normal rat showing L/S of normal cardiac muscle fibres. H & E 100x

c. Heart of Hypercholesterolemia induced rat showing separated cardiac muscle fibres, fatty infiltration (F), dilated vessels (DV). H & E 100x

d. Heart of Hypercholesterolemia induced rat shows separation of cardiac muscle fibres and deposition of fat inbetween (F). H & E 100x

e. Heart of High fat diet + *Eclipta alba* treated rat showing normal cardiac muscle fibres. H & E 100x

f. Heart of high fat diet + Clofibrate, treated rat showing normal architecture. H & E 100x
usually diagnostic. In normal, the sequence of changes is always the same. Each sinus impulse initiating atrial depolarisation (P wave) is followed by ventricular depolarisation (QRS complex) and ventricular repolarisation (T Wave). In analysing the ECG, the rate, rhythm and frontal plane, GRS axis should first be noted. Normal ECG of rat resembles that of a man (Larry Patrick Titley, 1992).

In the present study, the ECG pattern of the control rats (group 1) showed a regular, rhythmic sinus beat with the PQRST complex within the normal range. The average ‘P’ wave deflection is 0.05 mV, T wave 0.025 mV and heart rate 420 beats/mt.

The group II rats i.e., normal rats treated with Eclipta alba, there is no apparent deviation from the normal recording.

In group III rats subjected to hyperlipidemia, there was a significant elevation of ‘P’ value of 0.075 mV with ‘T’ wave amplitude of 0.05 mV. The R wave showed a very significant increase in amplitude as 0.275 mV which is more than double the normal value. This denotes a deviation in the path of electrical conduction. Further there is a slight though insignificant elevation of ST segment suggesting that the heart is under ischemic tension with a possibility of cellular damage (Schamorth, 1986). This is further revealed by bradycardia.
ECG Pattern of control and experimental animals

Control rat

Normal rat treated with Eclipta alba

Hypercholesterolemia induced rat

High fat diet + Eclipta alba

High fat diet + Clofibrate
In group IV high fat diet fed animals treated with *Eclipta alba*, the heart rate has picked up and has come to near normal. The configuration of PQRST pattern is also nearly normal.

Group V, high fat diet fed rats treated with clofibrate showed no significant change from normal recording, denoting complete recovery of the heart musculature and conductivity from the induced pattern.

This investigation throws open the possibility of further studies with *Eclipta alba* as it has shown beneficial effect upon hyperlipidemic hearts.

Figure 1 shows the changes in body weight (both initial and final) of experimental animals. Normal growth rate was observed in control (group 1) and *Eclipta alba* treated (group 2) rats. In high fat diet fed animals (group 3), the body weight was found to be increased significantly when compared to group I animals. The percentage increase in body weight of animals in group IV and group V are comparatively less when compared to group III.

Table 1 depicts the levels of Hemoglobin, RBC, WBC and platelet count and ESR of the experimental animals. No significant changes were observed in these parameters in *Eclipta alba* treated control animals (group II). A significant increase was noticed in Hb, RBC count, WBC count, platelet count and Erythrocyte sedimentation rate in group III animals. These levels were found to be decreased significantly in group IV (*Eclipta alba* + high fat diet) and group V (high fat diet + clofibrate treated) when compared to group III animals.
Figure 1  Changes in body weight of the control and experimental animals

Group I - Control, Group II - Eclipta alba control, Group III - Hypercholesterolemia induced, Group IV - High fat diet + Eclipta alba, Group V - High fat diet + clofibrate

Values are expressed as mean ± SD for 6 animals in each group

P values : *P < 0.05, **P < 0.01, ***P < 0.001 NS – Non significant
Table 1: Haemoglobin, Red blood cell count, White blood cell count, Platelet count and Erythrocyte sedimentation rate of control and experimental animals

Values are expressed as mean ± SD for 6 animals in each group

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I</th>
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<th>Group III</th>
<th>Group IV</th>
<th>Group V</th>
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</thead>
<tbody>
<tr>
<td>Haemoglobin (g/dl)</td>
<td>10.15 ± 0.19</td>
<td>10.05 ± 0.18&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>10.86 ± 0.09&lt;sup&gt;***&lt;/sup&gt;</td>
<td>10.37 ± 0.18&lt;sup&gt;***&lt;/sup&gt;</td>
<td>10.29 ± 0.07&lt;sup&gt;***&lt;/sup&gt;</td>
</tr>
<tr>
<td>RBC count (millions/cu.mm)</td>
<td>5.50 ± 0.38</td>
<td>5.58 ± 0.40&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>7.20 ± 0.50&lt;sup&gt;***&lt;/sup&gt;</td>
<td>6.40 ± 0.63&lt;sup&gt;'&lt;/sup&gt;</td>
<td>6.00 ± 0.23&lt;sup&gt;***&lt;/sup&gt;</td>
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<tr>
<td>WBC count (cells/cu.mm)</td>
<td>7140.00 ± 473.2</td>
<td>6970.40 ± 463.2&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>8564.00 ± 862.6&lt;sup&gt;***&lt;/sup&gt;</td>
<td>7802.60 ± 760&lt;sup&gt;***&lt;/sup&gt;</td>
<td>7529.80 ± 518.6&lt;sup&gt;***&lt;/sup&gt;</td>
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<tr>
<td>Platelet count (10&lt;sup&gt;6&lt;/sup&gt; cells/cu.mm)</td>
<td>2.00 ± 0.31</td>
<td>2.10 ± 0.32&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>2.60 ± 0.18&lt;sup&gt;**&lt;/sup&gt;</td>
<td>2.30 ± 0.23&lt;sup&gt;'&lt;/sup&gt;</td>
<td>2.20 ± 0.20&lt;sup&gt;***&lt;/sup&gt;</td>
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<td>Erythrocyte sedimentation rate (mm/hour)</td>
<td>1.40 ± 0.09</td>
<td>1.48 ± 0.18&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>2.40 ± 0.17&lt;sup&gt;***&lt;/sup&gt;</td>
<td>2.00 ± 0.14&lt;sup&gt;**&lt;/sup&gt;</td>
<td>1.50 ± 0.10&lt;sup&gt;***&lt;/sup&gt;</td>
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</table>

Group I - Control, Group II - *Eclipta alba* control, Group III - Hypercholesterolemia induced, Group IV - High fat diet + *Eclipta alba*, Group V - High fat diet + clofibrate.

Statistically significant variations are compared as follows:

<table>
<thead>
<tr>
<th>Group I</th>
<th>Vs</th>
<th>Group II</th>
<th>P values</th>
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<tbody>
<tr>
<td>Group I</td>
<td>Vs</td>
<td>Group III</td>
<td>* P &lt; 0.05</td>
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<td>Group III</td>
<td>Vs</td>
<td>Group IV</td>
<td>** P &lt; 0.01</td>
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<tr>
<td>Group III</td>
<td>Vs</td>
<td>Group V</td>
<td>*** P &lt; 0.001</td>
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<td>NS Non significant</td>
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There is close relationship between cardiovascular risk factors and haemorheology (Chien et al., 1987). Ernst et al. (1986) have postulated that both atherogenesis and blood rheology might have some common denominator. Adsorption phenomena taking place on surfaces which are in contact with blood plasma might constitute such a common denominator. These phenomena are a basic mechanism of early atherogenic changes and are the cause for abnormalities in blood rheology. Thus rheological variables might be an accessible marker for early hypercholesterolemia induced atherosclerotic properties. We have observed significant increase in hemoglobin content, hematocrit and RBC count in hypercholesterolemic rats. Clofibrate and Eclipta alba administered along with high lipid diet, restricted this change in hematological parameters. A positive correlation was observed between blood viscosity and parameters like hematocrit, fibrinogen, globulin as well as total lipid concentration by Schabitz et al., 1983.

Tarasov in 1976, reported an increase in number of erythrocytes, haemoglobin content, reticulocyte quantity, hematocrit indices, mass of circulating erythrocytes and intensity of erythropoiesis in myocardial infarction.

The changes observed in hyperlipidemic conditions could be due to the circulatory impairment of blood to the heart resulting in hypoxia, which stimulates erythrocytosis, which can lead to hemolysis. This in turn leads to increase in erythropoiesis which is a compensatory mechanism of oxygen insufficiency (Kok, 2000).
Significant increase in WBC count (Table 1) in group III hypercholesterolemic rats is in accordance with the report of Grylewski et al. (1971). Epidemiologic studies have shown correlation between WBC count and risk of atherosclerosis. Elevation of WBC count is associated with adverse outcome such as reduced epicardial blood flow, myocardial perfusion, thrombin resistance, high incidence of congestive heart failure and death (Baron, 2000). WBC plays a crucial role in the rheologic properties under stress-including stress of ischemia, enhancing their rheologic importance to participate in endothelial injury both acutely and chronically by adhering to the compounds and proteolytic enzymes (Ernst 1987). Cannon et al. (2001) have suggested that WBC count can serve as a new inexpensive tool for risk stratification in acute coronary syndromes.

Clofibrate and Eclipta alba supplementation (Group IV and V) is beneficial in maintaining almost normal levels of WBC in the blood (Table 1).

Recent studies have revealed that the plasma cholesterol concentrations are an influencing factor in RBC membrane cholesterol content, which in turn may regulate RBC membrane oxygen transport, RBC O₂ release and cellular O₂ availability (Buchwald et al., 2000).

In another study in rabbits fed with cholesterol diet for 2 months, fibrinogen and Willebrandt factor increased progressively, in intimal atherosclerotic lesion in aorta with increasing cholesterol levels. WBC and ferritin were unaffected. Increased levels of fibrinogen and von willebrandt factor known coronary risk factors, are strongly associated with the formation
of atherosclerotic plaques in rabbits. The plaques contain a considerable amount of fibrinogen related antigen (Hernandez et al., 2000).

Epidemiological studies have shown that the hemostatic parameters fibrinogen, factor VII, factor VIII, von willebrandt factor, tissue plasminogen activator, are risk factors markers of ischemic cardiovascular diseases. Ferritin and leucocyte have also been implicated.

Hypercholesterolemic rats (group III) showed significant increase in erythrocyte sedimentation rate, when compared to control (group I). An accelerated ESR is favoured by elevated levels of fibrinogen and to a lesser extent by globulins. These plasma factors cause an increase in rouleaux formation which due to more weight sediment rapidly than do single cells (Kalashnikova et al., 1997). Administration of Eclipta alba and clofibrate maintained the levels of ESR to near normal in group IV & V rats which could be due to their free radical scavenging activities.

The influence of oxidised LDL on blood cell functions play a role in the progression of atherosclerosis. The binding of oxidised LDL to the platelet surface leads to a modification of membrane fluidity, thus mediating the activating action of LDL on platelets. Both effects were proportional to the extent of lipid oxidation in LDL. So mildly oxidised LDL plays a crucial role in platelet activation. Clofibrate and Eclipta alba co-treatment prevented the increase in all the hematological parameters analysed.
Table 2 presents the levels of blood glucose and urea, total protein, uric acid and creatinine in serum, of control and experimental animals. No significant variation in these levels were noticed in group II animals, when compared to group I control animals, indicating the non-toxic nature of the drug. In group III high fat diet fed animals, a slight increase in the levels of urea, creatinine, total protein and glucose were noted but the increase is not significant when compared to control (group I) whereas a significant increase was recorded in these animals for uric acid levels. There is no significant change in these parameters (urea, creatinine, total protein and glucose) in group IV (High fat diet + Eclipta alba) and group V (high fat diet + clofibrate) animals. The uric acid levels were found to be lowered in group IV and V animals respectively when compared to group III animals.

Uric acid is the end product of purine metabolism and is a powerful scavenger of singlet $O_2$, peroxyl radicals and $OH^*$ radicals and so uric acid functions as an antioxidant. *In vivo*, reaction of $OH^*$ with uric acid produces a range of carbon centered radicals that mostly react with $O_2$ to give urate peroxyl radicals.

$$
\begin{align*}
R & \rightarrow \quad C-H + OH^* \\
& \rightarrow \quad R \rightarrow \quad C + H_2O \\
& \rightarrow \quad C + O_2 \\
& \rightarrow \quad R \rightarrow \quad CO_2^-
\end{align*}
$$
Table 2: Levels of Blood urea, Uric acid, Creatinine, Total protein in serum and Blood glucose in control and experimental animals. Values are expressed as mean ± SD for 6 animals in each group.

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<tr>
<td>Urea (mg/dl)</td>
<td>29.68 ± 2.39</td>
<td>30.96 ± 3.48&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>28.08 ± 2.73&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>31.32 ± 1.90&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>30.3 ± 2.9&lt;sup&gt;NS&lt;/sup&gt;</td>
</tr>
<tr>
<td>Uric acid (mg/dl)</td>
<td>1.40 ± 0.36</td>
<td>1.03 ± 0.19&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>2.15 ± 0.31&lt;sup&gt;&lt;u&gt;'&lt;/u&gt;&lt;/sup&gt;</td>
<td>1.81 ± 0.03&lt;sup&gt;'&lt;/sup&gt;</td>
<td>1.72 ± 0.08&lt;sup&gt;'&lt;/sup&gt;</td>
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<td>Creatinine (mg/dl)</td>
<td>1.14 ± 0.16</td>
<td>1.20 ± 0.12&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>1.17 ± 0.06&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>1.20 ± 0.07&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>1.19 ± 0.05&lt;sup&gt;NS&lt;/sup&gt;</td>
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<td>Total protein (g/dl)</td>
<td>5.15 ± 0.56</td>
<td>5.39 ± 0.14&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>5.92 ± 0.55&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>5.89 ± 0.53&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>5.84 ± 0.5&lt;sup&gt;NS&lt;/sup&gt;</td>
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<td>Glucose (mg/dl)</td>
<td>90.37 ± 10.69</td>
<td>94.91 ± 13.07&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>104.73 ± 12.81&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>97.08 ± 11.48&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>96.00 ± 12.07&lt;sup&gt;NS&lt;/sup&gt;</td>
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<td>Group V</td>
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These urate derived radicals although much reactive than OH\(^{-}\) are not completely harmless. They can inactivate enzymes like alcohol dehydrogenase and \(\alpha\)-antiproteinase. Thus uric acid, like GSH or ascorbate is not always a perfect antioxidant.

The increase in uric acid levels observed in Group III high lipid diet fed rats for 30 days could be due to accelerated nucleic acid breakdown caused by hyperlipidemia and not due to renal damage as serum urea and creatinine, markers of renal function remain normal, and increased serum uric acid levels are considered as potential risk factor for coronary heart disease (Persky, et al., 1979). Clofibrate co-treatment prevents the increase in the levels of uric acid. Our observations are consistent with the previous studies with fibrate on hyperlipidemic patients (Farnier et al., 1994). Similar results were obtained with \textit{Eclipta alba} co-treatment. 30 days supplementation of \textit{Eclipta alba} or clofibrate did not exhibit any significant change in the general biochemical parameters such as serum urea creatinine, glucose, and total protein.

The levels of total lipids, total cholesterol, free cholesterol, ester cholesterol, phospholipid, triacylglycerol and free fatty acids in serum, liver and heart of control and experimental animals are presented in the Figures 2,3 and 4 respectively. A slight but definite decrease was observed in serum lipid profile of group II animals when compared to the control rats, with a significant decrease in total lipids, ester and free cholesterol levels. The levels of these parameters were found to be increased in serum as well as in liver and heart tissues in group III animals in comparison with control rats. All
Figure 2  Levels of Total Lipids, Total cholesterol, Free cholesterol, Ester cholesterol, Phospholipid, Triacylglycerol and Free fatty acids in serum of control and experimental animals

Groups

Group I - Control, Group II - *Eclipta alba* control, Group III - Hypercholesterolemia induced, Group IV - High fat diet + *Eclipta alba*,
Group V - High fat diet + clofibrate
Group I Vs Group II; Group I Vs Group III; Group III Vs Group IV; Group III Vs Group V
Figure 3  Levels of Total Lipids, Total cholesterol, Free cholesterol, Ester cholesterol, Phospholipid, Triacylglycerol and Free fatty acids in liver of control and experimental animals

Groups

- Group I - Control
- Group II - *Eclipta alba* control
- Group III - Hypercholesterolemia induced
- Group IV - High fat diet + *Eclipta alba*
- Group V - High fat diet + clofibrate

Group I Vs Group II; Group I Vs Group III; Group III Vs Group IV; Group III Vs Group V

Values are expressed as mean ± SD for 6 animals in each group. P values: **P < 0.01, ***P < 0.001 NS - Non significant
Figure 4  Levels of Total Lipids, Total cholesterol, Free cholesterol, Ester cholesterol, Phospholipid, Triacylglycerol and Free fatty acids in heart of control and experimental animals

- Total Lipids
- Total Cholesterol
- Free Cholesterol
- Ester Cholesterol
- Phospholipid
- Triacylglycerol
- Free fatty acids

Groups:
- Group I - Control, Group II - Eclipta alba control, Group III - Hypercholesterolemia induced, Group IV - High fat diet + Eclipta alba, Group V - High fat diet + clofibrate
- Group I Vs Group II; Group I Vs Group III; Group III Vs Group IV; Group III Vs Group V
- Values are expressed as mean ± SD for 6 animals in each group. P values: * P < 0.05, ** P < 0.01, *** P < 0.001 NS - Non significant
these parameters were decreased in group IV and group V animals when compared to group III animals.

Table 3 shows the levels of cholesterol and triacylglycerol in lipoprotein fractions in serum of control and experimental animals. There is significant increase in serum HDL cholesterol and decrease in LDL cholesterol in group II animals when compared to group I control rats. Group III animals showed significant decrease in HDL cholesterol and increase in LDL and VLDL cholesterol when compared to normal controls. Both *Eclipta alba* and clofibrate supplemented in groups IV & V respectively, exhibited significant increase in HDL cholesterol and decrease in LDL and VLDL cholesterol levels.

The ratios for LDL-cholesterol to HDL-cholesterol and total cholesterol to HDL-cholesterol are presented in Figure 5. The ratios were marginally altered in group II animals when compared to control rats. There is a significant increase in these ratios in group III animals in comparison with the control rats and these elevations were altered on *Eclipta alba* co-treatment and clofibrate co-treatment in group IV and group V animals respectively.

From our study on lipid profile, it can be seen that cholesterol feeding with high fat diet in rats, resulted in hypertriglyceridemia, hyperphospholipidemia, hypercholesterolemia, increase in total cholesterol/HDL cholesterol and LDL cholesterol/HDL cholesterol ratios, elevated free fatty acids and total lipids, in the serum. Similar observations have been reported in experimental atherosclerotic animals (Stange *et al.*, 1975; Soleiko,
Table 3: Levels of Cholesterol and Triacylglycerol in lipoprotein fractions in serum of control and experimental animals. Values are expressed as mean ± SD for 6 animals in each group.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Lipid (mg/dl)</th>
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<th>Group III</th>
<th>Group IV</th>
<th>Group V</th>
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<tr>
<td>Lipoprotein</td>
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<tr>
<td>HDL</td>
<td>Cholesterol</td>
<td>24.19 ± 2.37</td>
<td>28.00 ± 1.5</td>
<td>16.02 ± 1.59</td>
<td>29.81 ± 1.82</td>
<td>28.16 ± 1.69</td>
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<td></td>
<td>Triacylglycerol</td>
<td>7.00 ± 0.67</td>
<td>6.90 ± 0.66NS</td>
<td>13.12 ± 1.02</td>
<td>8.80 ± 0.63</td>
<td>8.50 ± 0.84</td>
</tr>
<tr>
<td>LDL</td>
<td>Cholesterol</td>
<td>38.26 ± 2.41</td>
<td>34.00 ± 2.00</td>
<td>50.06 ± 4.16</td>
<td>40.28 ± 2.81</td>
<td>44.31 ± 2.86</td>
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<tr>
<td></td>
<td>Triacylglycerol</td>
<td>24.92 ± 1.93</td>
<td>22.90 ± 1.91NS</td>
<td>38.42 ± 3.51</td>
<td>29.63 ± 2.24</td>
<td>26.00 ± 2.61</td>
</tr>
<tr>
<td>VLDL</td>
<td>Cholesterol</td>
<td>17.10 ± 1.41</td>
<td>16.00 ± 1.42NS</td>
<td>22.25 ± 2.2</td>
<td>18.16 ± 1.42</td>
<td>17.43 ± 1.92</td>
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<tr>
<td></td>
<td>Triacylglycerol</td>
<td>40.36 ± 3.80</td>
<td>38.30 ± 3.78NS</td>
<td>51.38 ± 4.12</td>
<td>44.58 ± 3.9</td>
<td>42.00 ± 4.63</td>
</tr>
</tbody>
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<tr>
<td>* P &lt; 0.05</td>
<td>** P &lt; 0.01</td>
<td>*** P &lt; 0.001</td>
<td>NS Non significant</td>
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Figure 5 Ratio of LDL_{c}/HDL_{c} and Total c/HDL_{c}

Groups

Group I - Control, Group II - Eclipta alba control, Group III - Hypercholesterolemia induced, Group IV - High fat diet + Eclipta alba, Group V - High fat diet + clofibrate

Values are expressed as mean ± SD for 6 animals in each group
1974). There is direct correlation between cholesterol, triacylglycerol and serum LDL levels and severity of atherosclerosis (Murray and Tweddle, 1975).

Concomitant with the increases in serum lipid levels (Figure 2) tissue total lipids also increase significantly in liver and heart (Figure 3 & 4). Tissue cholesterol concentrations are also enhanced.

In the classical model of atherosclerosis provided by high fat diet fed animals, massive storage of cholesterol occurs in various organs. High fat diet also results in increased cholesterol deposition in the liver. Chernysheva, (1976) reported an increase in phospholipids and FFA in the heart of alimentary atherosclerotic dogs. Sphingomyelin fraction of phospholipid exhibit the greatest increase in atherosclerotic aorta (Kunnert and Krug, 1972) with resultant increase in Sphingomyelin/lecithin ratio and increased entry of sphingomyelin into the intima media of the vessel wall in cholesterol fed rabbits. Sharma et al. (1995) found an accumulation of cholesterol, phospholipids and triacylglycerol in liver, heart and aorta of experimental hypercholesterolemic rabbits. Our observations presented in Figures 3&4 provide evidence for uniform lipid accumulation in hepatic and heart tissues due to hypercholesterolemia.

The increase in lipid components observed in the tissues of untreated experimental animals may not be due to 8 synthesis from carbohydrate components but the origin of accumulating lipids may be from fat tissue (Gilmiyarova, 1969).
From Figures 2, 3 & 4, it is clear that both *Eclipta alba* and clofibrate lower lipid and cholesterol levels in liver and heart of treated animals. Both clofibrate and *Eclipta alba* lower not only cholesterol levels, but also triacylglycerol, FFA and phospholipid levels in serum as well as in liver and heart tissues. This lowering may be due to two possible mechanisms (a) the triacylglycerol already accumulated may be getting metabolised at a faster rate due to the drugs (b) the drugs may inhibit further synthesis of fatty acids and triacylglycerol from acetate or both. The decreased triacylglycerol levels observed in the liver and heart in group V rats may be due to increased lipoprotein lipase activity reported in clofibrate treated normal and atherosclerotic rats and / or due to clofibrate induced inhibition of lipolysis. (Decoopmann *et al.*, 1977). *Eclipta alba* is effective in lowering the serum cholesterol and lipid levels as well as in preventing their accumulation in liver and heart of experimental group IV rats. *Eclipta alba* extract may play an important role in lipid metabolism by increasing the bile flow (Kumar and Tirupathi, 1987). Clofibrate is known to inhibit VLDL synthesis. In addition it promotes the breakdown of VLDL to give IDL and LDL in the sequence leading to lowering of VLDL and IDL. This lowering of VLDL may be accelerated by inhibition of FFA turnover or FFA synthesis (Merlas *et al.*, 1996).

**HDL** has a protective action on the heart in the prevention of coronary heart disease (Fruchard and Duriez, 1998) and is a catabolic product of VLDL and LDL. The increase in serum HDL levels in *Eclipta alba* administréed normal and high lipid diet fed rats is a positive signal for *Eclipta alba* to be
considered as a potent anti-atherosclerotic drug. However, further investigations should be made on *Eclipta alba* mediated HDL turnover in atherosclerosis.

The hypolipidemic action of clofibrate can be attributed to its effect on various metabolisms. It interferes with sterol synthesis, inhibiting cholesterol synthesis at pre and post mevalonic sites (Gould *et al.*, 1967; White, 1971; Capuzzi, *et al.*, 1975). It inactivates or degrades the key enzymes in cholesterol synthesis, HMG CoA reductase (Kaneko *et al.*, 1977). In addition, clofibrate enhances the capacity of liver to oxidise cholesterol (Kritchevsky *et al.*, 1969; Rawat, 1975). Further it enhances fecal elimination of cholesterol as neutral sterols (Grundy *et al.*, 1972; Sodhi *et al.*, 1973) and stimulate tissue lipoprotein lipase activity in atherosclerotic rats (Decoopman *et al.*, 1976).

It is very interesting to note that *Eclipta alba* shows marginal but definite lowering of serum total lipids, total cholesterol, TG and free fatty acids in normal rats and is effective in controlling these levels in serum, liver and heart in rats fed with high fat diet (Group IV).

Understanding cellular and molecular responses to dietary fat are essential for developing new therapies for lipid related disorders such as heart disease, hypertension, diabetes and stroke.

The increase in cellular fatty acids appear to be one of the causes of myocardial injury during ischemia. According to Prasad *et al.* (1988), clofibrate feeding increases peroxisomal catalase and β-oxidation of fatty acids. It was
effective in lowering the free fatty acid levels of plasma and myocardium which appears to provide beneficial effects to the myocardium during ischemia.

Our observations on the effect of clofibrate on ECG and free fatty acid levels (Figures 2,3 & 4) in cholesterol fed rats are consistent with the above report.

"Because fatty acid metabolism is an important part of all these conditions (heart disease, diabetes, hypertension, stroke etc.). We must learn how the body responds to dietary fat, only then can we modulate these responses in a beneficial way" (Forman, 1997). There are cellular receptors that act as sensors of fatty acids. These sensors (PPAR alpha and PPAR delta) bind to fatty acids, then promote their breakdown by stimulating the activity of genes responsible for fat metabolism (Desvergne et al., 1999).

When there is abundance of fatty acids in the body (as in our study in hypercholesterolemic conditions), the excess lipids bind to the sensor which instructs cells to step up the breakdown process resulting in maintenance of normal fatty acid levels (Delerive et al., 2000).

Drugs like ciprofibrate (fibrates) that are currently used as serum triacylglycerol lowering agents also exert their effect through binding to the fatty acid sensor. But fibrates the most useful drugs are only effective at very high levels (Gervois et al., 1999). Eclipta alba is as effective as clofibrate in preventing the increase in triacylglycerol and free fatty acid levels in high lipid fed rats (Figure 2) and further studies with this drug may provide valuable
information about its mechanism of action and it may prove to be a much more potent agent for the treatment of hypercholesterolemic conditions.

The activities of AST, ALT, CPK, LDH, ALP and ACP in serum, liver and heart of control and experimental animals are reported in the Figures 6, 7 and 8 respectively. There is no significant alterations in the activity of these enzymes in group II animals when compared to control rats. This shows that there is no toxicity in *Eclipta alba*. A significant elevation in the activities of AST, ALT, CK, ALP and ACP is seen in serum and liver of group III animals when compared to group I animals. A significant decrease in the activity of LDH was recorded in serum of group III animals whereas a significant increase in the activities of these enzymes was observed in liver tissues. The alterations in the activities of these enzymes were correspondingly changed in group IV and group V animals in comparison with group III animals. In heart, activities of AST, ALT, CK and LDH were found to be reduced significantly in group III animals when compared to group I animals with a significant elevation in the activities of ACP and ALP. The corresponding changes were altered in group IV and group V animals approaching near normal levels.

A number of determinations of enzyme activity has been carried out for many years. These have fairly close relationship to a particular organ or tissue and so have a relatively high degree of specificity. When some disease process leads to increased cell breakdown in an organ, enzymes may escape in greater quantity with consequent increase in their activity in plasma. The pattern of change in circulation is specific for a particular organ in a particular disease condition and is of diagnostic value.
Figure 6  Activities of Aspartate transaminase, Alanine transaminase, Creatine kinase, Lactate dehydrogenase, Alkaline phosphatase and Acid phosphatase in serum of control and experimental animals

Group I - Eclipta alba control, Group III - Hypercholesterolemia induced, Group IV - High fat diet + Eclipta alba, Group V - High fat diet + clofibrate

Group I Vs Group II; Group I Vs Group III; Group III Vs Group IV; Group III Vs Group V

Values are expressed as mean ± SD for 6 animals in each group. P values: *P<0.05, ** P < 0.01, *** P < 0.001, NS - Non significant
Figure 7 Activities of Aspartate transaminase, Alanine transaminase, Creatine kinase, Lactate dehydrogenase, Alkaline phosphatase and Acid phosphatase in liver of control and experimental animals

Group I - Control, Group II - Eclipta alba control, Group III - Hypercholesterolemia induced, Group IV - High fat diet + Eclipta alba, Group V - High fat diet + clofibrate

Group I Vs Group II; Group I Vs Group III; Group III Vs Group IV; Group III Vs Group V

Values are expressed as mean ± SD for 6 animals in each group. P values: *P<0.05, ** P < 0.01, *** P < 0.001, NS - Non significant
Figure 8 Activities of Aspartate transaminase, Alanine transaminase, Creatine kinase, Lactate dehydrogenase, Alkaline phosphatase and Acid phosphatase in heart of control and experimental animals.

Groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>Control</td>
</tr>
<tr>
<td>Group II</td>
<td><em>Eclipta alba</em> control</td>
</tr>
<tr>
<td>Group III</td>
<td>Hypercholesterolemia induced</td>
</tr>
<tr>
<td>Group IV</td>
<td>High fat diet + <em>Eclipta alba</em></td>
</tr>
<tr>
<td>Group V</td>
<td>High fat diet + clofibrate</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD for 6 animals in each group. P values: ** P < 0.01, *** P < 0.001, NS - Non significant.
Abnormal fat metabolism often results in deranged carbohydrate metabolism, since these two compartments of intermediary metabolism are closely inter-related and it is a corollary that there should be an etiological connection between atherosclerosis and carbohydrate metabolism.

LDH is an important glycolytic enzyme regulating intracellular ratio of NAD/NADH. Increase in anaerobic forms enable more NAD to be produced from NADH. This regenerated NAD is available for triose phosphate dehydrogenase step of glycolysis and facilitates the subsequent step in the substrate linked phosphorylation of ADP to ATP which is utilised for synthetic reaction.

In our studies we have observed a significant decrease in serum LDH after 30 days of cholesterol feeding. Clofibrate and Eclipta alba treatment prevented this decrease to certain extent.

In tissues we have observed an increase in LDH activity in liver and decrease in heart in 30 days of cholesterol feeding. Eclipta alba supplement prevents the changes and maintains normal hepatic and myocardial LDH activities in high lipid diet fed rats.

Rylnikov (1974) reported that in atherosclerotic rabbits, there is decreased glycolysis and O₂ utilisation in most tissues except brain. Studies in our lab have showed anaerobic forms of LDH in serum and tissues in hypercholesterolemic animals which is an adaptive mechanism to tissue hypoxia that occurs secondary to hyperlipemia which is a major change
encountered in cholesterol fed rabbits due to tissue injury and change in membrane permeability (Johanna, 1978).

The decline in overall activity of this dehydrogenase in serum in Group III rats may be due to metabolic injury as a result of gross overloading of tissues by cholesterol. Liver is the centre of all metabolic functions of animal body and the increase in LDH found in group III animals may be due to an adaptive mechanism by the hepatocytes to counteract the, effect due to increased circulating levels of cholesterol and lipids.

The concentration of α-glycerophosphate is reduced in livers of clofibrate treated rats (Pereira and Holland, 1970). This may retard the overall glycolytic rate and clofibrate is seen to reduce the activity of different glycolytic enzymes in man and rats (Lufkin et al., 1974; Zakim et al., 1970). The detailed mechanism of the interaction of clofibrate with enzyme activities is still unknown.

Our observations are consistent with the above reports. Eclipta alba is effective in preventing the changes due to atherogenic diet.

The lowering of glycolytic enzymes in heart tissues in group III animals may be due to inhibition of G6PD resulting in accumulation of G6P which inhibits hexokinase and glycolysis, which has been reported earlier (Gordon et al., 1977). The accumulated G6P may stimulate the activity of G6Pase and the glucose formed takes up alternate pathways such as sorbitol and glucuronic acid pathways of metabolism. Moreover, in ischemic conditions, the
heart exhibits decreased LDH activities with increase in serum LDH (Bora & Srivastava, 1985). Consistent with the above report, the observed decrease in LDH in myocardium may be due to hypercholesterolemia induced ischemic heart (ECG & Plate 3).

Lysosomes, a group of acid hydrolytic enzymes, having a specific subcellular localisation is part of the body's cellular scavenging system. Lysosomes respond to excess protein, lipid and mucopolysaccharide, by hydrolysing the material to more easily disposable units. Extracellular lysosomes in the vessel wall have been reported to play an important role in several vascular diseases and both extra and intracellular lysosomes may be directly involved in cellular lipid metabolism (Riede and Staubesand, 1977).

In our studies we have observed a significant increase in the lysosomal enzyme acid phosphatase in serum, liver and heart of untreated hypercholesterolemic rats. Clofibrate co-treatment lowers lysosomal acid phosphatase level in the atherosclerotic heart, liver and serum considerably (Figures 6,7 & 8). While *Eclipta alba* co-administration (Group IV) also decreases the elevated levels (Saxena et al., 1993). In normal control rats, *Eclipta alba* does not exhibit any marked variation.

The metabolic significance of increased lysosomal activities in hypercholesterolemic rats may reflect the activation of catabolic processes or due to tissue and membrane damage.
Another possible role of lysosomes is the uptake of extracellular materials by endocytosis (Axline and Cohn, 1970). In atherosclerotic animals, concentration of aortic cell cholesterol increases, most of the lipid arising from the plasma and in part through endogenous synthesis. The LDL, the principle plasma vehicle for cholesterol, enters the arterial wall. The increased levels of lysosomal enzymes may be associated with endocytosis by the smooth muscle cell, of the large amounts of lipid within the arterial wall (Halliwell, 1983).

In an atherosclerotic aorta, unlike normal aorta the cholesterol is intralysosomal (in normal the cholesterol is associated with plasma membrane). The extracellular lipid enters by way of pericystic vesicles which subsequently fuse with the lysosomes. The high stability of lysosomes are due to high cholesterol content as it has been shown that cholesterol can stabilise rat liver lysosomes in vitro (Wenzell et al., 1975).

According to Wenzell et al. (1975) with increasing serum cholesterol concentrations cholesterol within the lysosomes labilise the lysosomal membranes and disrupts them leading to leakage of enzymes in to the serum as observed in our studies.

The increase in acid phosphatase in serum and tissues of hypercholesterolemic rats may also be due to increased circulating levels of LDL which has been shown to activate lysosomal enzymes (El Saadany et al., 1991).
Recent studies have shown that 3 days to 9 months administration of diets containing phthalate or clofibrate in rats results in changes in a characteristic order commencing with alterations in the distribution of lipids within the liver, quickly followed by proliferation of hepatic peroxisome and induction of the specialised P-450 isoenzymes catalysing omega oxidation of fatty acids. There follows a phase of mild liver damage indicated by induction of glucose 6 phosphatase activity and a loss of glycogen, eventually leading to the formation of enlarged lysosomes through autophagy and accumulation of lipofuscin. Associated changes are found in kidney and thyroid. In kidney, the changes are limited to the tubules and is similar to the changes in liver. The effects on thyroid are more marked. There is even a hypothesis explaining the progress from the initial metabolic effects to final formation of liver tumors (Hinton et al., 1995).

The transaminases AST and ALT catalyse the reversible conversion of certain α-keto acids to amino acids. This transamination process is important in linking carbohydrate and amino acid metabolism and in clearly establishing the relationship between intermediates of TCA cycle and aminoacids.

A characteristic observation is change in tissue transaminases (Figures 7 & 8). Serum transaminases increase in hypercholesterolemic rats (Figure 6). High cholesterol diet alters free amino acid spectrum of blood and tissues, the changes result in increased amino acid levels for carbohydrate synthesis. Consequently, gluconeogenesis will be accelerated. Conflicting reports have been reported in serum and tissue transaminase activities in atherosclerosis (Maevski, 1969; Gilimiyarovua et al., 1975).
Consistent with the reports of El Saadany et al. (1991), we have also observed increase in the activities of AST, ALT, alkaline and acid phosphatase in the serum of high lipid diet fed untreated rats. Supplementation of Eclipta alba and clofibrate with high lipid diet maintained almost normal levels of the same in the serum. Chandra et al. (1987) have reported that Eclipta alba counteracts the increase in hepatic lipid peroxidation, serum AST and alkaline phosphate in CCl₄ induced liver damage in rats.

The percentage reduction in the levels of LDH, AST and CK in clofibrate treated rats is comparatively less when compared to Eclipta alba treated group IV. This may be due to the effect of clofibrate on the thyroid gland (Hattori et al., 1990).

Price et al. (1986) have reported that treatment of rats for periods of 3 days to 9 months or longer with the hypolipidemic drugs, clofibrate and fenofibrate or with a phthalate causes alterations in the thyroid. The colloid is shrunken and contains calcium rich inclusions. There is increase in size and number of lysosomes, hypertrophy of the golgi apparatus and dilation of the rough endoplasmic reticulum. These changes are consistent with persistent hyperactivity in the gland.

The biological role of alkaline phosphatase is still uncertain. In addition to its role in calcification it has been suggested that the enzyme participates in the synthesis of collagen and other fibrous proteins (Goldberg et al., 1960).
The increase in alkaline phosphatase found in atherosclerotic conditions may be due to an overload of normal transfer mechanism or due to repair mechanisms in the injured cells in the liver and heart tissues.

Creatine kinase is an important enzyme which catalyses the reversible phosphorylation of creatine to creatine phosphate. During muscle contraction the creatine phosphate breaks down to provide energy in the form of ATP. When there is muscular dystrophy or in muscle wasting there is leakage of the enzyme into the serum. The observed increased in serum in untreated hypercholesterolemic rats (Group III) may due to the damage caused by hyperlipidemia to heart tissue. Both clofibrate and Eclipta alba are beneficial in alleviating this damage as reflected by the levels of serum creatine phosphokinase in Group IV and Group V animals.

Table 4 depicts the levels of lipid peroxide and glutathione in serum, heart and liver of control and experimental animals. No significant alterations were found to be noted in group II animals when compared to group I animals which reflects the non-toxic nature of the drug. In both serum and the tissues (heart and liver), the levels of lipid peroxide was found to be elevated significantly with a concomitant decrease in the levels of glutathione in group III animals. These levels were retained at near normal values on co-treatment with Eclipta alba and clofibrate in group IV and group V animals respectively.

Figure 9 represents the levels of non-enzymic antioxidants such as ceruloplasmin, ascorbic acid and α-tocopherol in serum of control and experimental rats. There is no significant change in level of these antioxidants
Table 4: Levels of Lipid peroxide and Glutathione in serum, heart and liver of control and experimental animals. Values are expressed as mean ± SD for 6 animals in each group.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
<th>Group V</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum (nM of TBA reactants/ml)</td>
<td>3.37 ± 0.24</td>
<td>3.36 ± 0.23NS</td>
<td>5.65 ± 0.16***</td>
<td>3.44 ± 0.14***</td>
<td>3.40 ± 0.15***</td>
</tr>
<tr>
<td>Heart (nM of TBA reactants produced/mg protein)</td>
<td>3.55 ± 0.164</td>
<td>3.50 ± 0.160NS</td>
<td>5.99 ± 0.18***</td>
<td>3.63 ± 0.14***</td>
<td>3.61 ± 0.17***</td>
</tr>
<tr>
<td>Liver (nM of TBA reactants produced/mg protein)</td>
<td>40.04 ± 11.8</td>
<td>39.25 ± 11.6NS</td>
<td>60.83 ± 6.8***</td>
<td>42.16 ± 4.3**</td>
<td>48.16 ± 15.2***</td>
</tr>
<tr>
<td>Serum (mg/dl)</td>
<td>54.34 ± 2.5</td>
<td>52.6 ± 2.9NS</td>
<td>44.6 ± 2.8***</td>
<td>58.3 ± 3.5**</td>
<td>59.3 ± 2.0**</td>
</tr>
<tr>
<td>Heart (nM of GSH/g tissue)</td>
<td>4.29 ± 0.22</td>
<td>4.28 ± 0.18NS</td>
<td>3.2 ± 0.18***</td>
<td>4.40 ± 0.29***</td>
<td>4.34 ± 0.21***</td>
</tr>
<tr>
<td>Liver (nM of GSH/g tissue)</td>
<td>4.13 ± 0.38</td>
<td>4.10 ± 0.36NS</td>
<td>2.87 ± 0.30***</td>
<td>4.20 ± 0.20***</td>
<td>4.17 ± 0.25***</td>
</tr>
</tbody>
</table>

Group I - Control, Group II - Eclipta alba control, Group III - Hypercholesterolemia induced, Group IV - High fat diet + Eclipta alba, Group V - High fat diet + clofibrate.

Statistically significant variations are compared as follows:

- Group I Vs Group II
- Group I Vs Group III
- Group III Vs Group IV
- Group III Vs Group V

<table>
<thead>
<tr>
<th>P values</th>
<th>Group I Vs Group II</th>
<th>Group I Vs Group III</th>
<th>Group III Vs Group IV</th>
<th>Group III Vs Group V</th>
</tr>
</thead>
<tbody>
<tr>
<td>**</td>
<td>P &lt; 0.01</td>
<td>**</td>
<td>**</td>
<td>NS</td>
</tr>
<tr>
<td>***</td>
<td>P &lt; 0.001</td>
<td>**</td>
<td>**</td>
<td>NS</td>
</tr>
<tr>
<td>NS</td>
<td>Non significant</td>
<td>**</td>
<td>**</td>
<td>NS</td>
</tr>
</tbody>
</table>
Figure 9 Levels of Ceruloplasmin, Vitamin C and vitamin E in serum of control and experimental animals

Groups

- **Ceruloplasmin (Units/ml)**
- **Vitamin C (mg/100 ml)**
- **Vitamin E (mg/100 ml)**

Group I - Control, Group II - Eclipta alba control, Group III - Hypercholesterolemia induced, Group IV - High fat diet + Eclipta alba, Group V - High fat diet + clofibrate

Group I Vs Group II; Group I Vs Group III; Group III Vs Group IV; Group III Vs Group V

Values are expressed as mean ± SD for 6 animals in each group. P values: ** P < 0.01, *** P < 0.001, NS - Non significant
Figure 10  Activities of Glutathione reductase, Glutathione peroxidase, Glutathione-S-transferase, Superoxide dismutase and Catalase in hemolysate of control and experimental animals

- Glutathione reductase (micro gram of GSSG utilised/min/mg protein)
- Glutathione peroxidase (micro gram of GSH utilised/min/mg protein)
- Glutathione-S-transferase (nM of CDNB conjugated/min/mg protein)
- Superoxide dismutase (units/mg protein)
- Catalase (nM of H2O2 decomposed/min/mg protein)

Group I - Control, Group II - Eclipta alba control, Group III - Hypercholesterolemia induced, Group IV - High fat diet + Eclipta alba, Group V - High fat diet + clofibrate

Group I Vs Group II; Group I Vs Group III; Group III Vs Group IV; Group III Vs Group V

Values are expressed as mean ± SD for 6 animals in each group. P values: *** P < 0.001, NS - Non significant
Figure 11 Activities of Glutathione reductase, Glutathione peroxidase, Glutathione-S-transferase, Superoxide dismutase and Catalase in liver of control and experimental animals

Groups

- Glutathione reductase (micro gram of GSSG utilised/min/mg protein)
- Glutathione peroxidase (micro gram of GSH utilised/min/mg protein)
- Glutathione-S-transferase (nM of CDNB conjugated/min/mg protein)
- Superoxide dismutase (units/mg protein)
- Catalase (nM of H2O2 decomposed/min/mg protein)

Group I - Control, Group II - Eclipta alba control, Group III - Hypercholesterolemia induced, Group IV - High fat diet + Eclipta alba, Group V - High fat diet + clofibrate

Group I Vs Group II; Group I Vs Group III; Group III Vs Group IV; Group III Vs Group V

Values are expressed as mean ± SD for 6 animals in each group. P values: *P < 0.05, ** P < 0.01, *** P < 0.001, NS - Non significant
Figure 12  Activities of Glutathione reductase, Glutathione peroxidase, Glutathione-S-transferase, Superoxide dismutatse and Catalase in heart of control and experimental animals

Group I - Control, Group II - Eclipta alba control, Group III - Hypercholesterolemia induced, Group IV - High fat diet + Eclipta alba, Group V - High fat diet + clofibrate
Group I Vs Group II; Group I Vs Group III; Group III Vs Group IV; Group III Vs Group V
Values are expressed as mean ± SD for 6 animals in each group. P values: *P<0.05, ** P < 0.01, *** P < 0.001, NS - Non significant
in group II rats when compared to group I rats. A significant decrease was observed in group III animals for these antioxidants when compared to the controls and their levels were maintained at near normal values on coadministration of *Eclipta alba* and clofibrate in group IV and group V animals respectively.

The activities of enzymic antioxidants such as GRx, GPx, GST, SOD and CAT in hemolysate, liver and heart of control and experimental animals are recorded in Figures 10, 11 and 12 respectively. In both hemolysate and the liver and heart tissues, the activities of these enzymic antioxidants were found to be decreased significantly in group III animals when compared to the controls. No significant change was noticed for these enzymes in group II animals. The activity of all these enzymes were retained at almost near normalcy in group IV and group V animals respectively.

The origin of atherosclerosis is uncertain, but a popular current theory is that it begins with a damage by some mechanism to the vascular endothelium. This could be followed by attachment of monocytes from the circulation that develop into macrophages within the vessel wall. Activated monocytes and macrophage could injure neighbouring cells by secreting $O_2^-$, $H_2O_2$ and hydrolytic enzymes, and factors released by macrophages can stimulate the proliferation of smooth muscle cells. Macrophages also release platelet stimulating factors and adherence of platelets to injured endothelium could cause release of other agents that encourage proliferation of smooth muscle cells (Halliwell, 1989).
What roles could be played by oxidants in atherogenesis? First activation of macrophages or their monocyte precursors eg. in a fatty streak could injure neighbouring cells and lead to more endothelial damage and damage to smooth muscle cells.

Second, normal macrophages possess some LDL receptors but if LDL is peroxidised it is recognised by separate receptors known as the acetyl LDL receptors or the scavenger receptors. LDL bound to these receptors is taken up with enhanced efficiency so that cholesterol accumulates within the macrophages and may convert it into a foam cell. Arterial smooth muscle cells, endothelial cells and macrophages are capable of oxidising LDL so that macrophages internalise faster. Lysine residues of protein moiety of LDL are modified by peroxidation.

Third, any lipid peroxide in LDL may contribute to the initial endothelial cell damage. Then peroxidised LDL acts as chemotactic factors for blood monocytes encouraging their recruitment into an atherosclerotic lesion. Low concentrations of peroxides then accelerate cyclooxygenase and lipoxygenase catalysed reactions in the endothelium and in any platelets present, leading to enhanced formation of eicosanoids. Oxidised LDL may also stimulate the production of eicosanoids by macrophages. There have been several suggestions that oxidation products of cholesterol may also be involved in atherogenesis. Cholesterol is oxidised to a wide variety of products in peroxidising lipid systems and oxidised cholesterol is known to be toxic for arterial smooth muscle cells (Halliwell, 1983).
Lipid peroxidation is limited by very potent free radicals, which includes superoxide (O$_2^-$) and hydroxyl radicals (OH) and reactive molecule hydrogen peroxide (H$_2$O$_2$). Hydrogen peroxide and O$_2$ may be directly damaging or often interact to form a highly reactive species that can attack almost every molecule in living cells (Halliwell and Gutteridge, 1989).

It might therefore be suggested that elevated blood lipid concentrations (as observed in our studies in high lipid fed rats) could lead to elevated blood lipid peroxide concentrations (Table 4) contributing to endothelial injury and accelerating the whole process of atherogenesis.

In the present study, lipid peroxide levels were found to be elevated in serum, heart and liver of group III hypercholesterolemic animals. This increase in oxidants (Lipid peroxides) might be due to the decrease in the antioxidant levels (both enzymic and non-enzymic) in the system. This is confirmed from our observations in (Table 4) co-administration of Eclipta alba enhanced the antioxidant status of the animals may be by destroying the oxidants produced by hyperlipidemia, thereby restoring the levels of lipid peroxides to near normal (Group IV). In this study, the levels of lipid peroxides were maintained at near normalcy in Eclipta alba treated group IV animals. This could be attributed to the antioxidant nature and free radical scavenging property of Eclipta alba. Eclipta alba may react with peroxyl radicals strongly suggesting that, this drug has the potency to behave as an inhibitor of lipid peroxidation. (Chandra et al., 1987).
If oxidants initiate atherosclerosis and/or contribute to its pathology, then intake of antioxidants or drugs which improve antioxidant status may be expected to have beneficial effects.

Plasma contains powerful preventive and chain breaking antioxidants which limits lipid peroxidation and depletion or failure of these protective mechanisms is also involved in the pathogenesis of atherosclerosis.

From our studies we can observe that *Eclipta alba* has not only the capacity to limit the increases in lipid fractions in serum of high lipid diet fed rats but also improves the antioxidant status (Figures 10,11 & 12) suggesting that it could be a good choice as an antiatherogenic drug for extensive future studies.

Numerous enzymatic and non-enzymatic mechanisms protect the cell against oxidative injury. The removal of damaging products is catalysed by antioxidant enzymes (SOD, CAT, GPx, GST, GRx) and they play a major role in protecting the cell from oxidative damage caused by free radicals. Non-enzymatic defenses are provided by antioxidant vitamins A,C,E, ceruloplasmin, glutathione and selenium.

The cell is protected against damage by several mechanisms such as oxygen consumption, primary radical scavenging, release of bound endogenous reactors, inhibition of oxygen transport etc., (Halliwell *et al.*, 1996). Among the protectors, cellular thiols are an important group with glutathione (GSH) probably being the most important. GSH is a tripeptide (γ-glutamyl cysteinyl-
glycine) which exists intracellularly with proteins or non-protein sulphhydrils. Increase in lipid peroxides in hypercholesterolemic rats may also be due to decrease in the levels of GSH (Folhe, 1998). GSH is a powerful antioxidant and a free radical scavenger which resulted in the formation of reduced GSH and other disulfide upon action. Besides being a free radical scavenger and GSH is a powerful antioxidant, it plays a major role in restoring the other antioxidants to their reduced state. This aspect of GSH could be correlated with decrease in the levels of other antioxidants. Previous studies on cell membrane damage by ischemia due to hypercholesterolemia support our findings (Halliwell, 1977). *Eclipta alba* co-treatment prevented the decrease of glutathione by supplementing the antioxidants as well as by its free radical scavenging property thereby increasing the GSH levels. Clofibrate co-administration also increased the levels of GSH in group V rats.

Ceruloplasmin is an α-globulin protein that is synthesised in the liver and has a circulating half-life of 5-6 days in serum and can diffuse into the alveolar space. A number of investigators have previously showed that ceruloplasmin is vulnerable to oxidative attack, with ensuring loss of ferroxidase activity. The oxidative attack on ceruloplasmin by superoxide radicals and hydrogen peroxide can cause loss of ferroxidase activity. Eventhough the levels of ceruloplasmin are increased, the increased levels has only little impact on lipid peroxidation (Yamanak *et al.*, 1974).

In our present study, the levels of ceruloplasmin were found to be decreased in group III animals when compared to controls. This could be due to the increased peroxidation in hypercholesterolemic conditions which could
have resulted in the loss of ferroxidase activity of the ceruloplasmin. Hence, a depletion in the levels of ceruloplasmin, is observed in the present study (Hoffman, 1985) Eclipta alba and clofibrate co-treated rats showed significantly elevated levels of this antioxidant which might be due to their antioxidant nature.

Vitamins C & E are powerful antioxidants and free radical scavengers that inhibits lipid peroxidation (Van Poppel et al., 1993). Decreased plasma vitamin C concentrations are reported in smokers and may be a consequence of greater vitamin C turnover in response to sustained oxidant load rather than decreased dietary uptake (Botton Smith et al., 1991). The decrease in Vitamin C levels in hypercholesterolemia might be due to its consumption by scavenging radicals formed in hyperlipidemid condition (Group III). Eclipta alba co-treatment improved the antioxidant status thereby increasing the levels of vitamins C & E, hence, levels of these antioxidants were maintained at near normalcy in Eclipta alba treated (Group IV) and group V rats. Our studies show a similar trend with previous findings on the effect of vitamin E (Horvath, 1993).

The ability of oxidants such as H₂O₂ to induce DNA damage in cells, perhaps by site specific hydroxyl radical formation is of particular interest in relation to the mechanism of action of several peroxisome proliferators (carcinogens) (Halliwell, 1983). A wide range of compounds including drugs that lower blood lipid concentrations like clofibrate, produce enlargement of the liver accompanied by marked increase in the number of hepatic peroxisomes in several animal species. Peroxisomes not only increase in number, but their
overall balance of enzyme activities change. Thus the activity of peroxisomal system for β-oxidation of fatty acids often increase more than that of catalase (Halliwell, 1983).

Reddy et al. (1984) have suggested that tumors result because of excess H$_2$O$_2$ in peroxisomes (eg. due to increased-oxidation of fatty acids) cannot be fully metabolised by the catalase present and leaks out of these organelles. If some of the H$_2$O$_2$ survives to reach the nucleus, DNA damage could be expected. Perfusion of lauric acid, a substrate for peroxisomal β-oxidation in isolated rat livers from control rats produced no increase in efflux of oxidised glutathione from the liver. However if rats had been pretreated with a peroxisome proliferator, livers isolated from them respond to infusion of lauric acid by greatly increased GSSG efflux. Increased GSSG efflux due to H$_2$O$_2$ escape from peroxisomes increases its metabolism by glutathione peroxidase in the cytoplasm. Hypolipidemic agents also cause proliferation of hepatic endoplasmic reticulum and its associated cytochrome which might also be sources of O$_2^-$ and H$_2$O$_2$ in vivo (Mantha et al., 1996).

Glutathione acts as an antioxidant, by serving for several enzymes that reduce hydrogen peroxide and organic peroxides and by mediating the reduction of dehydro ascorbate and oxidised forms of α-tocopherol and other compounds. Glutathione functions directly in the destruction of hydrogen peroxide and lipid peroxides by providing a substrate for glutathione peroxidase. Glutathione level in the tissue is maintained normally by the enzyme glutathione reductase.
Group IV and V rats showed a decrease in the levels of glutathione in blood and heart. Free radicals liberated during the process of lipid peroxidation leads to the accumulation of GSSG, the oxidised product of GSH. Administration of our drug maintains the glutathione level to normal. Glutathione peroxidase is the main enzyme involved in scavenging free radicals and $H_2O_2$ in myocardial tissue. Under physiological conditions, superoxide dismutase in heart muscle converts the superoxide anions generated to hydrogen peroxide, which is acted upon by the enzyme catalase and glutathione peroxidase. Glutathione peroxidase is the enzyme present relatively in high levels in heart and glutathione-S-transferase eliminates toxic compounds by conjugating them with glutathione (Ishikawa, 1984).

There is significant decrease in the activity of glutathione peroxidase and glutathione-S-transferase in cholesterol administered rats which causes a depletion in the levels of glutathione which is a substrate for glutathione peroxidase and glutathione-S-transferase and this can attribute to the decrease in the enzyme activity. Drug treated rats show elevated glutathione peroxidase activity with increase in glutathione level. Eclipta alba due to its antioxidant nature, will detoxify the free radicals produced during lipid peroxidation and thus maintain the glutathione level.

The 2 key enzyme which play an important role in body defense mechanism against the harmful effects of oxygen free radicals are catalase and superoxide dismutase. A decrease in the activity of enzymes can cause production of toxic radicals which can disrupt the cardiac membrane (Samuelson, 1977).
A decrease in the activity of superoxide dismutase is seen in cholesterol fed rats. Superoxide dismutase was reported to contain arginine and histidine residues at its active site. Free radicals attach on these highly reactive amino acids can result in chemical modification of these amino acids with loss of activity. High cholesterol (hypercholesterolemia) generates free radicals, which damages myocardial cells and also modifies the amino acids at the active site of superoxide dismutase which results in loss of activity (Vaille, 1990).

The enzyme activity was retained in group IV, V rats. This could be due to antioxidant nature of drug Eclipta alba which prevents the formation of free radicals and hence protects the enzyme (Rashid et al., 1992). A decrease in the activity of heart catalase activity has been observed in hypercholesterolemic rats. A decrease in the catalase activity has been shown to be caused by hydrogen peroxide and superoxide radicals which are generated by the lipid peroxidising effects of Eclipta alba where as co-administration of Eclipta alba restored the enzyme activity (Wagner et al., 1986).

The results of our study reveals the non toxic nature of Eclipta alba to the liver and heart. Previous studies have shown Eclipta alba to be hepatoprotective (Mogri et al., 1986) and further long term study on Eclipta alba's renoprotective and hepatoprotective nature will confirm whether its use as a hypolipidemic agent is better than existing fibrates and other drugs which exhibit toxicity on long term administration.

Further, from our studies we can observe that Eclipta alba has not only the capacity to limit the increases in lipid fractions in serum of high lipid diet fed rats but also improves the antioxidant status suggesting that it could be a good choice as an antiatherogenic drug for extensive future studies.