

## Chapter VI

To study the comparative antioxidant and hypolipidaemic effect of aqueous extracts of *E. littorale* and herbal combination (*C. longa*, *E. officinalis*, *T. foenum-graecum*, *E. littorale*) in cholesterol fed rats.

- Introduction
- Experimental design
- Results
- Discussion
- Summary

## Introduction

Hyperlipidaemia has been implicated in atherosclerosis, which is the leading cause of death among world population. The prevalence of CAD in urban India (10%) is about double that of rural India (5%) and about 4-fold higher than in the US (2.5%) (Reddy, 1998). The excess burden of CAD in Indians is due to a combination of nature (genetic predisposition) and nurture (environmental or lifestyle factors) (Enas et. al., 1997). Decreased physical activity and increased consumption of calories and saturated fat result in abdominal obesity, insulin resistance, and atherogenic dyslipidaemia such as, low levels of HDL, high levels of LDL resulting in a high LDL/HDL ratio, along with abnormal concentrations of other lipoproteins. One of the most fascinating developments of the past ten years is the increasing evidence that the inexorable progress of atherosclerosis can be slowed, arrested, and even reversed by aggressive lipid-lowering therapy (Thompson, 1997). High cholesterol diet feeding increases serum LDL levels and oxidative stress which results in increase oxidized LDL levels and thereby increases atherosclerotic plaque formation (Warnholtz et. al., 2001). There has been a growing interest in studies that concern with the prevention of uncontrolled oxidative process leading to various diseases in living system. Large number of lipid lowering drugs like statins, bile acid sequestrants, nicotinic acid, fibric acid derivatives, etc. are available in the market which leads to hyperlipidaemia and thus decrease incidence of atherosclerosis. The above class of drugs have their own serious side effects when taken for a long duration. There are a lot of plants which shows hypolipidaemic and antioxidant property and have little toxicity or no toxicity and can be used as an alternate therapy against hyperlipidaemia.

As discussed in earlier chapters, there are many plant extracts used in treatment of diabetes which also demonstrated hypolipidaemic effect. Also large number of plants demonstrated lipid lowering effect in cholesterol fed rats. It has been observed that rats treated synchronously with hypercholesterolemic (HC) diet and curcumin, showed decreased cholesterol levels when compared to those kept on HC diet alone (Subba Rao et.al., 1970; Godkar et.al., 1996). Flavonoids from *Emblica officinalis* and *Mangifera indica* (Anila and Vijayalakshmi, 2002) and isolated fenugreek fractions with saponins and high fibre content (Madar and Stark, 2002), had also been reported to act as hypocholesterolemic agents in cholesterol fed rats. A systematic randomized clinical trials of herbal medicinal products used to lower serum cholesterol demonstrated significant reductions in total serum cholesterol levels of between 10% and 33% (Coon and Ernst, 2003). *C. longa*, *E. officinalis*, *T. foenum-graecum* and *E. littorale* are potent antioxidants as mentioned in previous chapters. There are no reports regarding hypolipidaemic effect of *E. littorale* in cholesterol fed rats or rats given atherogenic diet. A single report of its hypolipidaemic effect was reported by Murali et.al., 2002, in streptozotocin-induced diabetic rats. Present study was an attempt to evaluate the hypolipidaemic effect of aqueous extract of *E. littorale* in cholesterol fed rats and compare it with the herbal combination (*C. longa*, *E. officinalis*, *T. foenum-graecum* and *E. littorale*).

### **Experimental Design**

Male albino Charles foster rats were made hypercholesterolemic by feeding 1% cholesterol and 0.1% cholic acid with the standard diet, as mentioned in chapter II. Normal control rats were supplemented with stock diet instead of cholesterol and cholic

acid. The treated rats received aqueous extracts of *E. littorale* and herbal combination at a dose of 1.5 g dry plant equivalent extract/100g body weight respectively via gastric intubation for 6 weeks. Lovastatin, the HMG-CoA reductase inhibitor, was used as reference drug (2 mg/100g b. wt.). Change in body weight was monitored every week. Blood samples were collected at 0<sup>th</sup>, 3<sup>rd</sup> and 6<sup>th</sup> week and the animals were sacrificed at the end of the experiment and liver and kidney dissected out for estimation of various parameters as mentioned below. Rats were divided into five groups of six rats each, as follows:

- Group I : control rat fed with diet without cholesterol (NC).
- Group II : hypercholesterolemic rats which received hypercholesterolemic diet (HC).
- Group III : hypercholesterolemic rats which received hypercholesterolemic diet and *E. littorale* aqueous extract simultaneously (HC + EL).
- Group IV : hypercholesterolemic rats which received hypercholesterolemic diet and herbal combination aqueous extract simultaneously (HC + ALL).
- Group V : hypercholesterolemic rats which received hypercholesterolemic diet and lovastatin simultaneously (HC + L).

#### **Lipidaemic parameters**

Serum cholesterol, serum triglycerides and serum HDL cholesterol were estimated by using commercial kit method on 0<sup>th</sup>, 3<sup>rd</sup> and 6<sup>th</sup> week. From these results LDL cholesterol, VLDL and LDL/HDL ratio were calculated. Lipids were extracted by the procedure developed by Folch et.al., 1957, from liver and kidney after sacrificing the animal on 6<sup>th</sup> week. The dried lipid residues were dissolved in 1 ml of ethanol for

cholesterol and triglyceride assays by the same kit used for plasma lipid analysis. Hepatic HMG-Co A reductase activity was also estimated (Rao and Ramakrishnan, 1975).

### **Antioxidant parameters**

Catalase (CAT) and superoxide dismutase (SOD) activity and reduced glutathione (GSH) and lipid peroxidation (LPO) levels were estimated in erythrocyte, liver and kidney of treated rats and compared with that of untreated hypercholesterolemic rats.

### **Results**

Animals were treated with hypercholesterolemic diet along with EL alone and herbal combination of selected medicinal plants. After 6 week the normal control rats which were given diet without cholesterol showed 22% increase in body weight whereas hypercholesterolemic rats which were given high cholesterol diet showed 35% increase. Both the extract treated rats showed less increase in body weight, even after cholesterol feeding (Fig 1), which was even less than the NC group suggesting that the extracts were able to control the increase in body weight, where, herbal combination showed an increased efficacy. Similarly, liver and kidney weight in both the extract treated groups were maintained even after cholesterol feeding (Fig 2, 3).

Cholesterol fed rats showed increased levels of serum cholesterol, triglycerides and decreased HDL cholesterol levels as compared to rats fed on normal diet. But *E. littorale* (EL) and herbal combination (ALL) administration simultaneously along with the hypercholesterolemic diet was able to check the increase in serum cholesterol (Fig 4), serum triglycerides (Fig 5) and amicably increased the HDL cholesterol (Fig 6) levels on 3<sup>rd</sup> and 6<sup>th</sup> week of administration. The extracts also significantly decreased serum LDL cholesterol (Fig 7), VLDL cholesterol (Fig 8) and the atherogenic index (LDL/HDL)

ratio (Fig 9) as compared to untreated hypercholesterolemic rats. Liver and kidney cholesterol levels in untreated hypercholesterolemic rats showed significant increase as compared to normocholesterolemic rats, which was the same in liver and triglyceride levels also. *E. littorale* (EL) and herbal combination (ALL) administration to hypercholesterolemic rats significantly decreased the liver and kidney cholesterol as well as triglyceride levels respectively (Fig 10 – 13). Estimation of hepatic HMG-Co A reductase activity showed a significantly decreased activity in *E. littorale* and herbal combination treated hypercholesterolemic rats (Fig 14).

Hypercholesterolemic rats showed an increased activity in erythrocyte CAT, SOD activities, increased LPO and decreased GSH levels as compared to normocholesterolemic rats. The activities of CAT and SOD were significantly decreased along with decreased LPO levels and increased GSH levels in *E. littorale* and herbal combination extract treated hypercholesterolemic rats (Fig 15 – 18). Though there was an increase in activity of liver CAT and SOD activity in hypercholesterolemic rats, no such effect was seen in kidney CAT or SOD activities, though a slight increase was seen which was not statistically significant. Moreover there was an increase in LPO and decrease in GSH levels in liver and kidney of hypercholesterolemic rats. These changes were almost brought to normal levels in extract treated hypercholesterolemic rats (Fig 19 – 26).

## **Discussion**

Hypercholesterolemia is considered a major risk factor in the progression of coronary atherosclerosis and is associated with an increase in the incidence of myocardial ischemia and cardiac events (Smith et.al., 1992). According to classis studies, reducing

the total cholesterol to less than 200 mg/dl and the low-density lipoprotein (LDL) to less than 160 mg/dl reduces the risk for heart disease (Chen et.al., 1991). Since, many herbal medicinal products have potential hypocholesterolemic activity and encouraging safety profiles, in the present study aqueous extracts of *E. littorale* and herbal combination has been evaluated for hypocholesterolemic effect in cholesterol fed rats.

This is a first case report of hypolipidaemic effect of *E. littorale* in cholesterol fed rats. There was a significant increase in body weight of HC rats as compared to NC group, due to supplemented cholesterol and cholic acid in the diet, but were controlled in extract treated HC rats. This is supported by the observations in lipid profile, where there was a decrease in elevated levels of serum cholesterol, triglycerides, LDL and VLDL cholesterol in extract treated HC rats as compared to untreated HC rats. *E. littorale* was reported to show hypolipidaemic effect in streptozotocin-induced NIDDM rats (Murali et.al., 2002) and was also observed in diabetic patients as reported in chapter III of this thesis. Curcumin, the phenolic yellowish pigments of *C. longa* showed lipid-lowering potency in rats fed high fat diet, probably due to alterations in fatty acid metabolism (Asai and Miyazawa, 2001). *Emblica officinalis* when administered to cholesterol-fed rabbits showed a decrease in serum cholesterol, TG, phospholipid and LDL levels and tissue lipid levels (Mathur et.al., 1996; Anila and Vijayalakshmi, 2002). Hypercholesterolemic rats when fed with an ethanol extract from defatted fenugreek (*Trigonella foenum-graecum*) seeds showed reductions in plasma and liver cholesterol levels (Stark and Madar, 1993). A unique fibre cocktail of fenugreek seed powder, guar gum and wheat bran (Fibernat) administration resulted in reduction in cholesterol content of liver and increased clearance of circulating atherogenic LDL and VLDL. The results

suggest that Fibernat's effect on plasma LDL concentration is also possibly mediated by increased receptor-mediated catabolism of VLDL (Venkatesan et.al., 2003). There are also reports of various polyherbal formulation on cholesterol fed rats, such as Caps HT2, containing the methanolic extracts of selected parts of nine plants, where the oral administration of the formulation, significantly raised HDL cholesterol levels along with significant reduction in the atherogenic index and body weight and also showed lipid peroxidation inhibition and superoxide and hydroxyl radicals scavenging properties (Mary et.al., 2003). Yet another herbal formulation – Liposem – demonstrated hypolipidaemic and antioxidant activity by significantly raising HDL cholesterol and the HDL/LDL and VLDL+LDL ratio. The atherogenic index and the reduction in body weight were also significant, indicating the effectiveness against hyperlipidaemia and obesity and were also found to scavenge hydroxyl and superoxide free radicals (Mary et.al., 2002).

Since oxidation of LDL plays a significant role in atherogenesis, amelioration of oxidative stress is equally important as controlling or decreasing dyslipidemia. In the present study, as demonstrated in chapter V, *E. littorale* and herbal combination showed good antioxidant effect. A significant decrease in CAT and SOD activity and LPO levels and an increase in GSH levels in *E. littorale* and herbal combination treated groups has been demonstrated in erythrocyte, liver and kidney. Again, herbal combination showed an increased efficacy as seen in the results due to the constituents present and its varied antioxidant mechanism of action as mentioned earlier. LDL is known as a “bad cholesterol” as it transfers cholesterol from the liver to circulation. There was significant increase in serum LDL levels when in untreated HC group, which was decreased in

extract treated HC group. In fact, extract treated group showed no significant increase in LDL cholesterol levels and levels remain almost equal to NC group at 6<sup>th</sup> week. The results suggest that EL and ALL might be affecting at LDL receptor's upregulation or gene transcription level and thereby facilitating removal of cholesterol from the circulation as demonstrated by earlier mentioned "Fibernat" (Venkatesan et.al., 2003). LDL/HDL ratio has direct correlation with the cardiovascular disease risk as an increase in the ratio is directly proportional to increased risk. As compared to untreated HC group at 6<sup>th</sup> week, all the treated groups showed significant decrease in the ratio and it was almost similar to the NC group.

HMG CoA reductase is the key enzyme for cholesterol synthesis. It converts HMG CoA to mevalonate and in the present study HMG CoA/Mevalonate ratio was measured. The ratio is inversely proportional to HMG CoA reductase activity i.e. an increase in ratio indicates decreased activity. Untreated HC group showed a slight increase in HMG CoA/Mevalonate ratio as compared to NC group at 6<sup>th</sup> week. This showed feedback inhibition of HMG CoA reductase activity in HC group by exogenous cholesterol feeding. All the treated groups showed further decrease in HMG CoA reductase activity and the effect was seen more in herbal combination (HC + ALL) and were comparable to Lovastatin (HC + L) treated rats and thus suggesting a possible interaction with the enzyme and thus lowering cholesterol levels. Such an effect had been demonstrated by flavonoids isolated from *E. officinalis* in hypercholesterolemic rats (Anila and Vijayalakshmi, 2002).

Apart from these mechanism, the cholesterol lowering effect could be due to increased elimination; in fact, curcumin significantly elevated the activity of hepatic

cholesterol-7 alpha-hydroxylase, thus stimulated the conversion of cholesterol to bile acids, an important pathway of elimination of cholesterol from the body, in hypercholesterolemic rats (Srinivasan and Sambaiah, 1991). *E. officinalis* treated hypercholesterolemic rabbits excreted more cholesterol and phospholipids, suggesting that the mode of absorption was affected (Mathur et.al., 1996). And fenugreek seeds contained hypocholesterolaemic components which appear to be saponins that interact with bile salts in the digestive tract inhibiting taurocholate and deoxycholate absorption (Stark and Madar, 1993). Comparing *E. littorale* and herbal combination, the later showed increased efficacy, which may be due the potent components of the combination and its varied principles and mechanism of action as described above.

Thus, *E. littorale* was reported to show antioxidant and hypolipidaemic effect in cholesterol fed rats for the first time. Combining the selected medicinal plants was able to potentially increase the antioxidant and hypolipidaemic efficacy and thus paving way for a combinatorial therapy in controlling dyslipidemia caused by dietary cholesterol.

### **Summary**

Aqueous extracts of *E. littorale* and herbal combination to cholesterol fed rats for a period of 6 weeks was able to control body weight gain and increase in liver and kidney weight and also significantly reduced serum cholesterol, triglycerides, LDL and VLDL cholesterol along with significantly increasing HDL cholesterol levels.

Both the extracts were also able to decrease liver and kidney cholesterol and triglyceride levels in hypercholesterolemic rats.

Hepatic HMG CoA reductase activity inhibition by both the extracts demonstrated as one of the possible mechanism of cholesterol lowering mechanism.

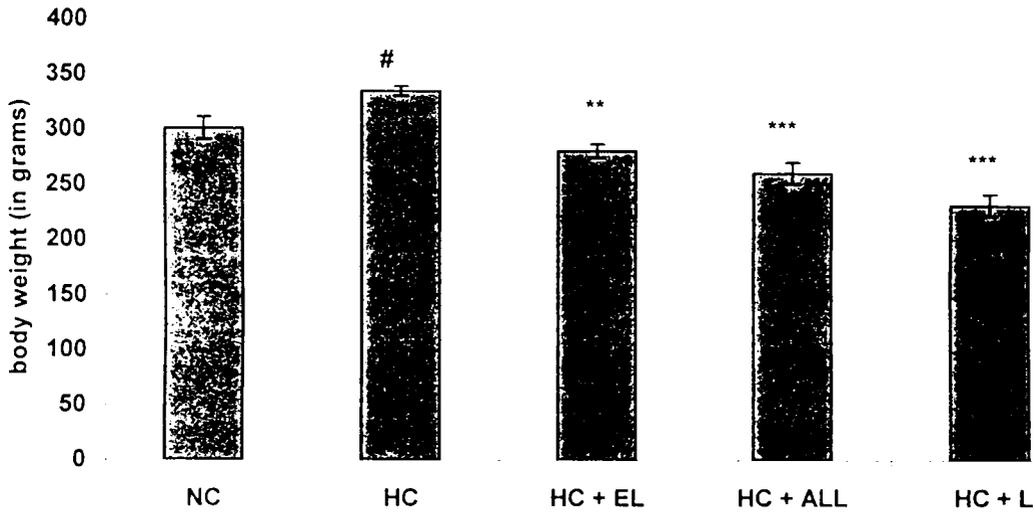
*E. littorale* and herbal combination decreased CAT, SOD activity and LPO levels and increased GSH levels in erythrocyte and liver of treated HC rats as compared to untreated HC rats. Kidney CAT and SOD activity did not show any significant change in activities in NC, untreated HC or treated HC rats but decreased LPO levels and increased GSH levels were observed in treated HC rats as compared to untreated HC rats.

In all the observations herbal combination showed increased efficacy than *E. littorale* and the results were somewhat comparable to the results of the reference drug, Lovastatin.

Herbal combination, thus showed an increased efficacy in normalizing dyslipidemia and oxidative stress caused by cholesterol feeding in rats.

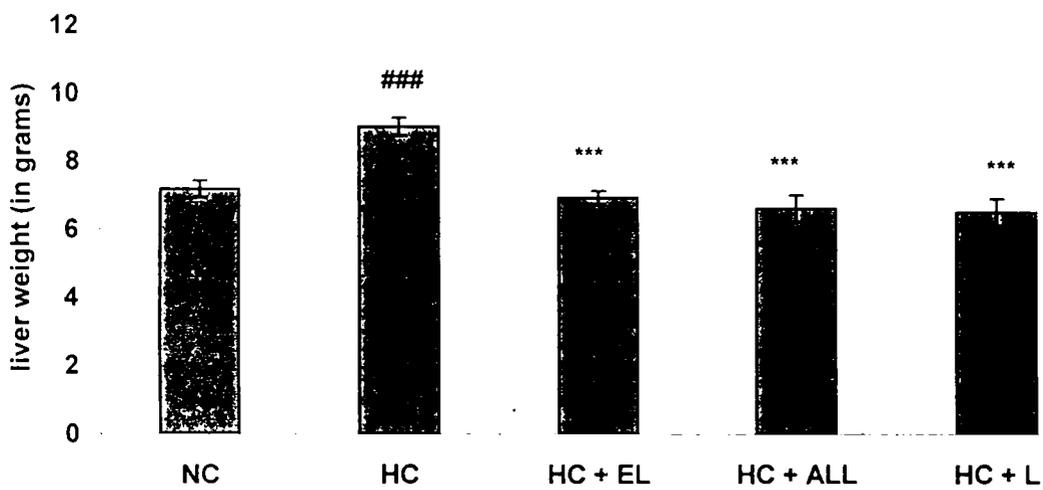


Fig 1: Effect of aqueous extracts of *E. littorale* (EL) and herbal combination (ALL) on body weight in cholesterol fed rats at 6th week



# P < 0.05 as compared to NC at 6th week  
\*\* P < 0.01, \*\*\* P < 0.001 as compared to HC at 6th week

Fig 2: Effect of aqueous extracts of *E. littorale* (EL) and herbal combination (ALL) on liver weight in cholesterol fed rats



\*\*\* P < 0.001 as compared to HC at 6th week  
### P < 0.001 as compared to NC at 6th week

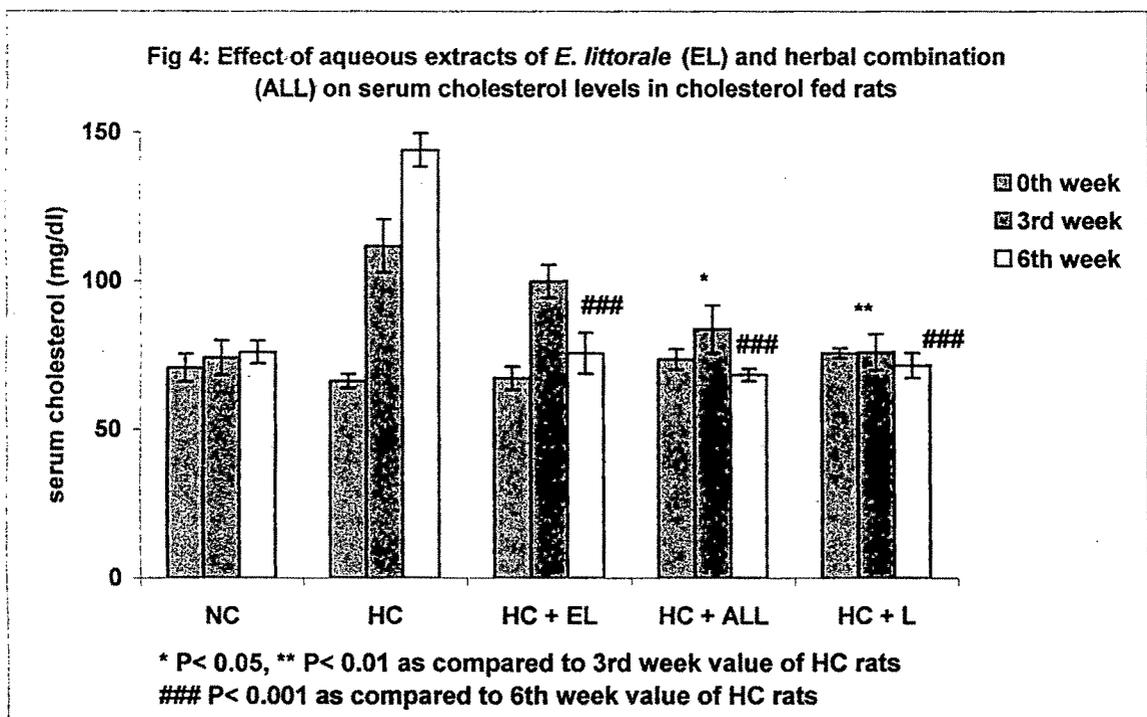
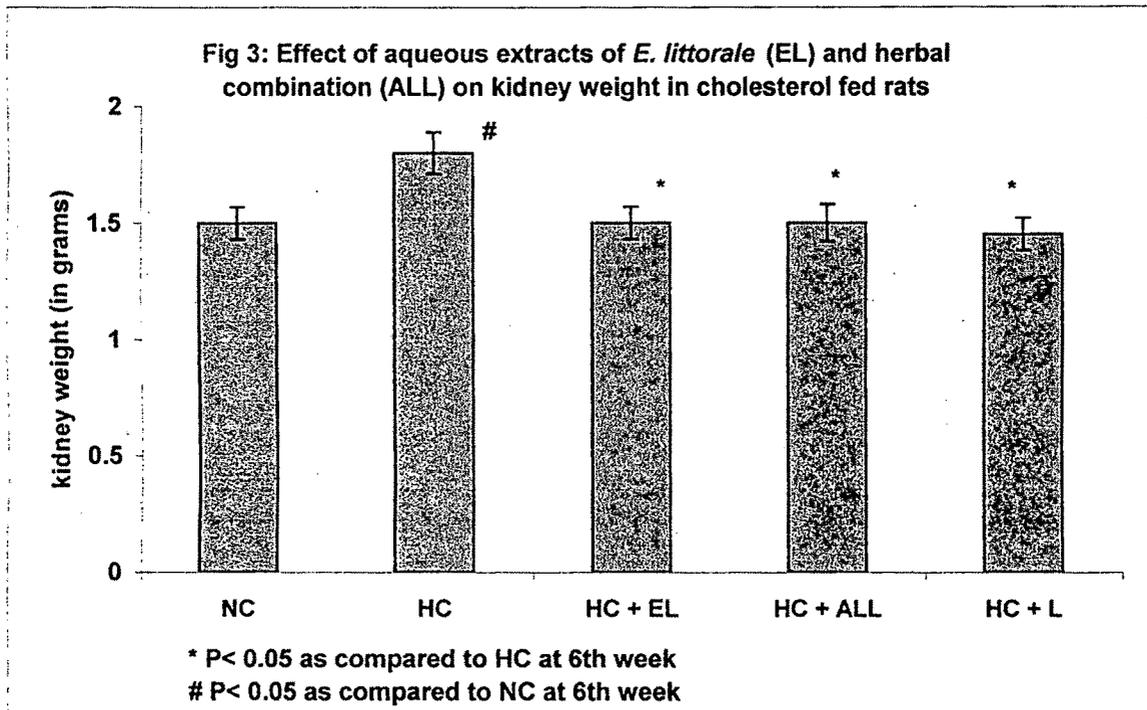
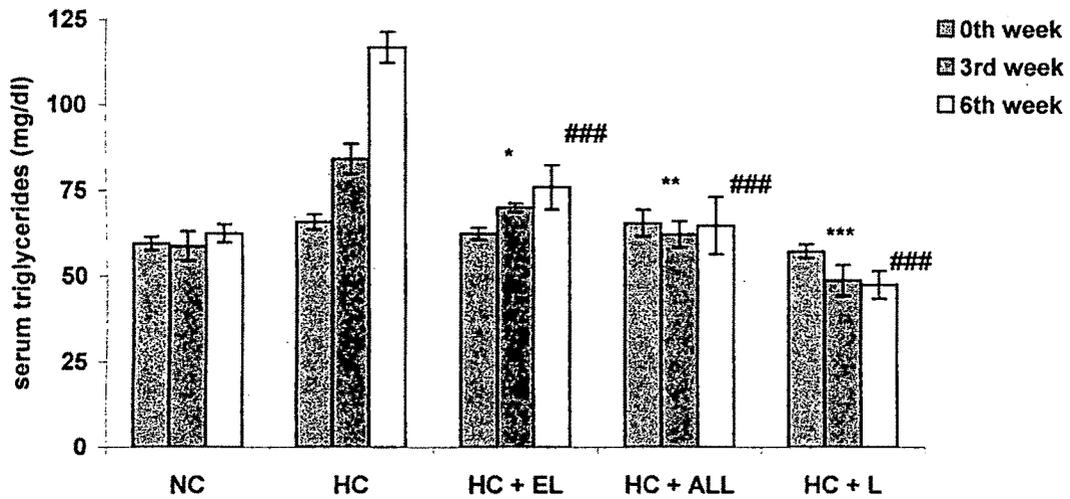
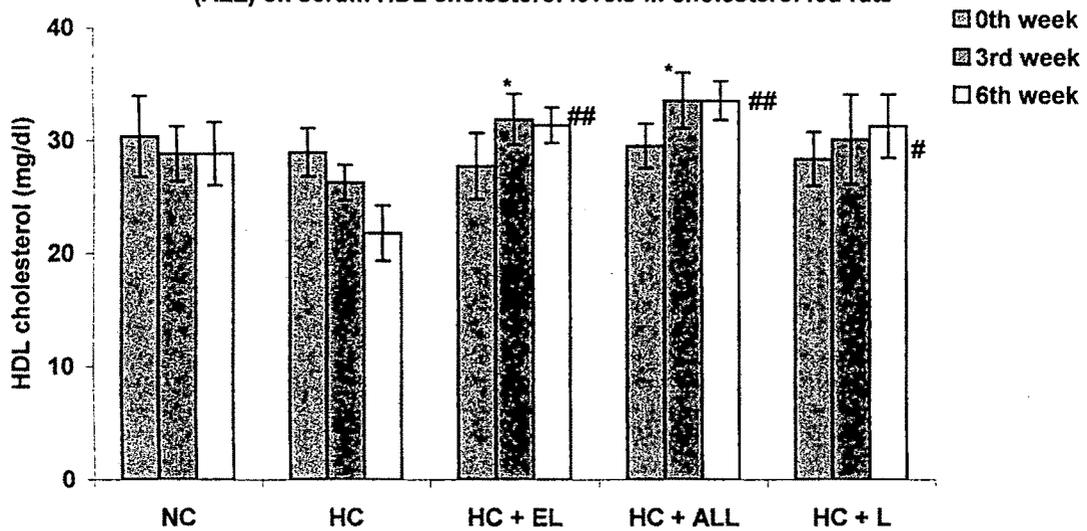


Fig 5: Effect of aqueous extracts of *E. littorale* (EL) and herbal combination (ALL) on serum triglycerides levels in cholesterol fed rats

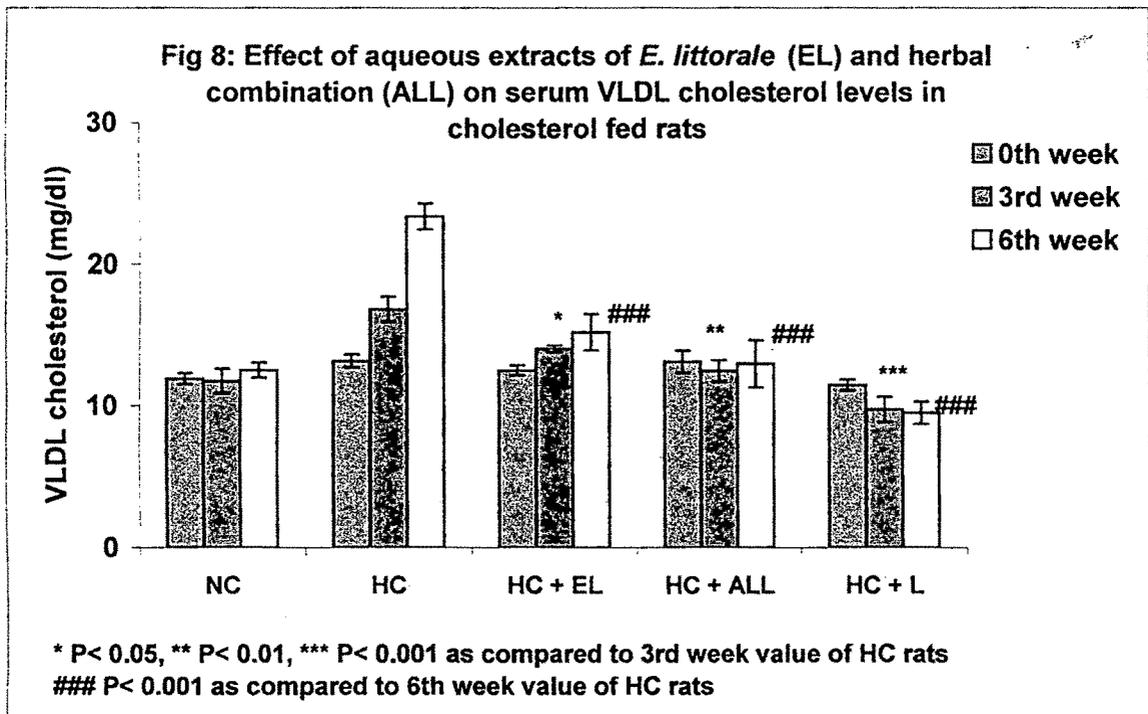
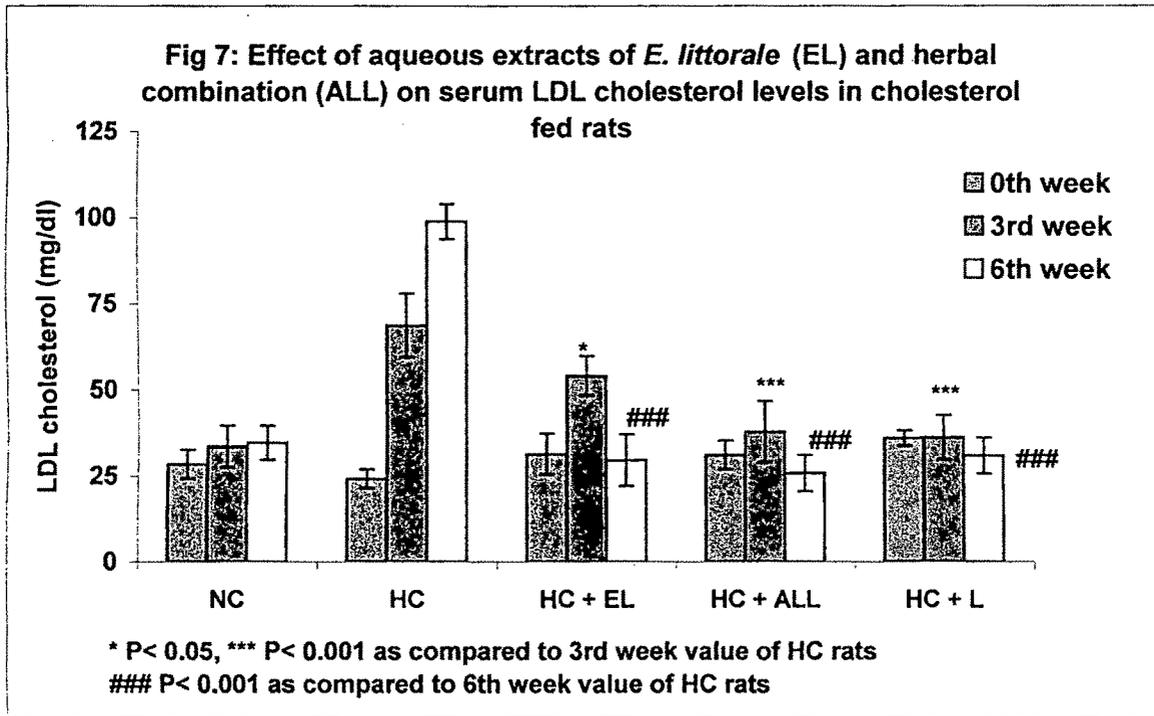


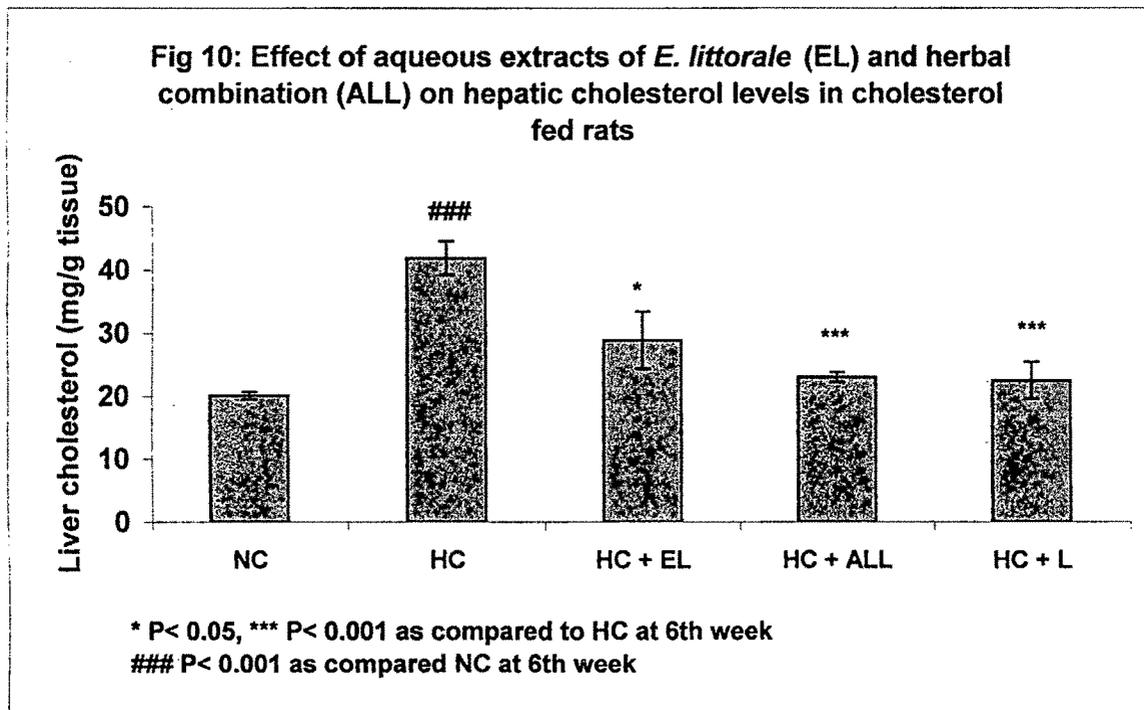
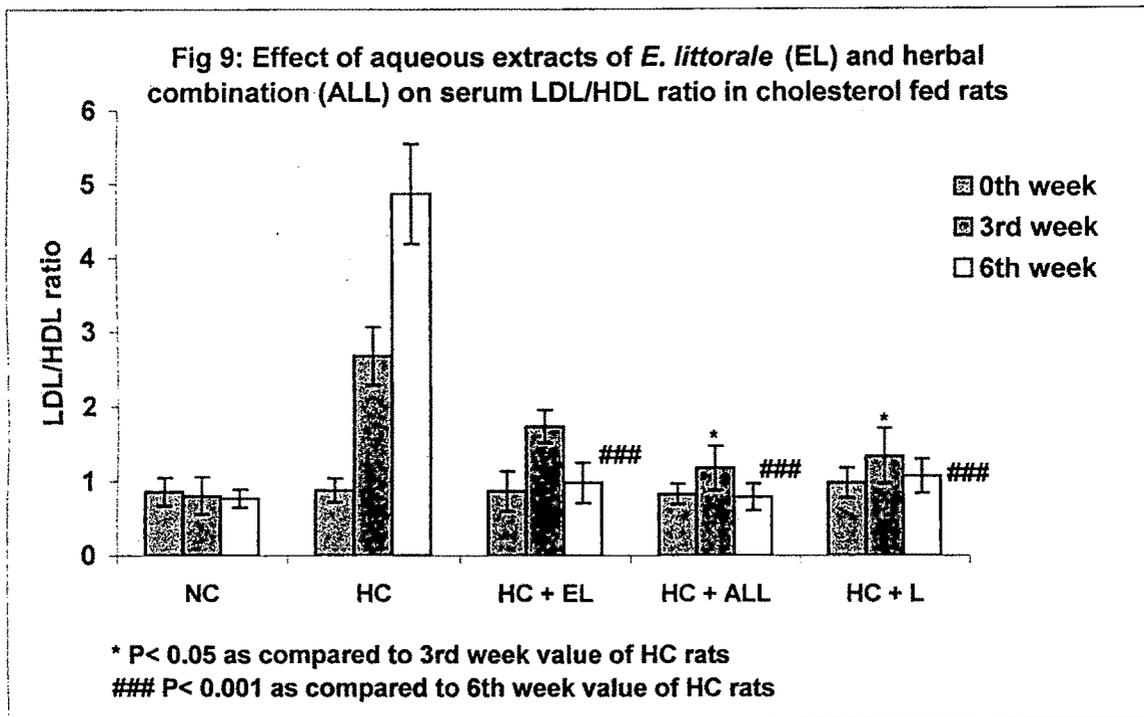
\* P < 0.05, \*\* P < 0.01 as compared to 3rd week value of HC rats  
 ### P < 0.001 as compared to 6th week value of HC rats

Fig 6: Effect of aqueous extracts of *E. littorale* (EL) and herbal combination (ALL) on serum HDL cholesterol levels in cholesterol fed rats

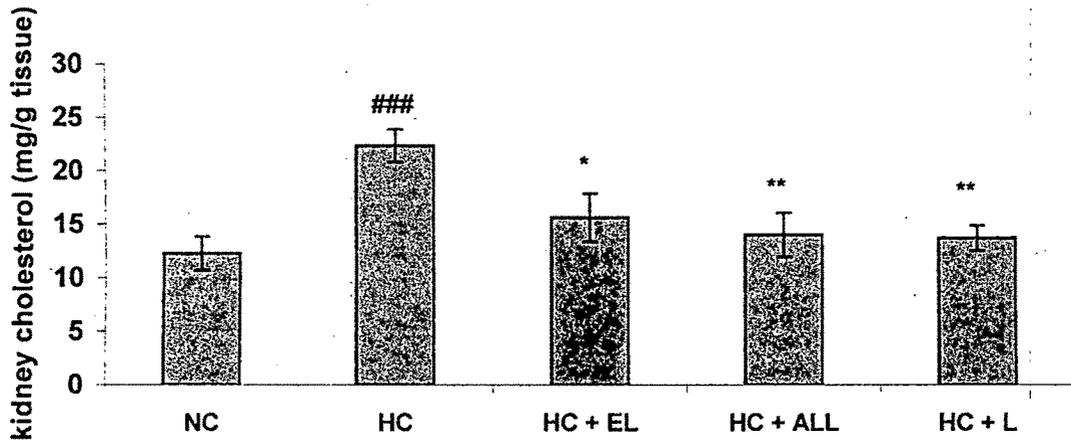


\* P < 0.05 as compared to 3rd week value of HC rats  
 # P < 0.05, ## P < 0.01 as compared to 6th week value of HC rats



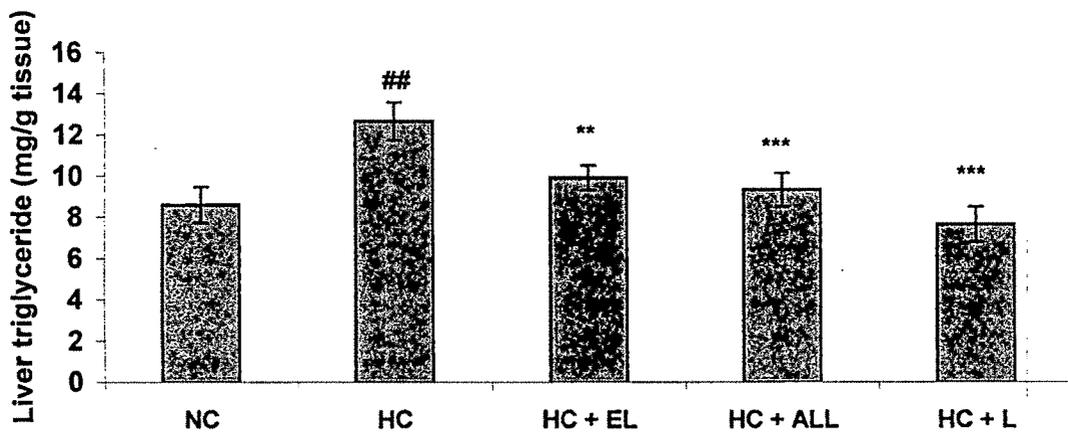


**Fig 11: Effect of aqueous extracts of *E. littorale* (EL) and herbal combination (ALL) on renal cholesterol levels in cholesterol fed rats**



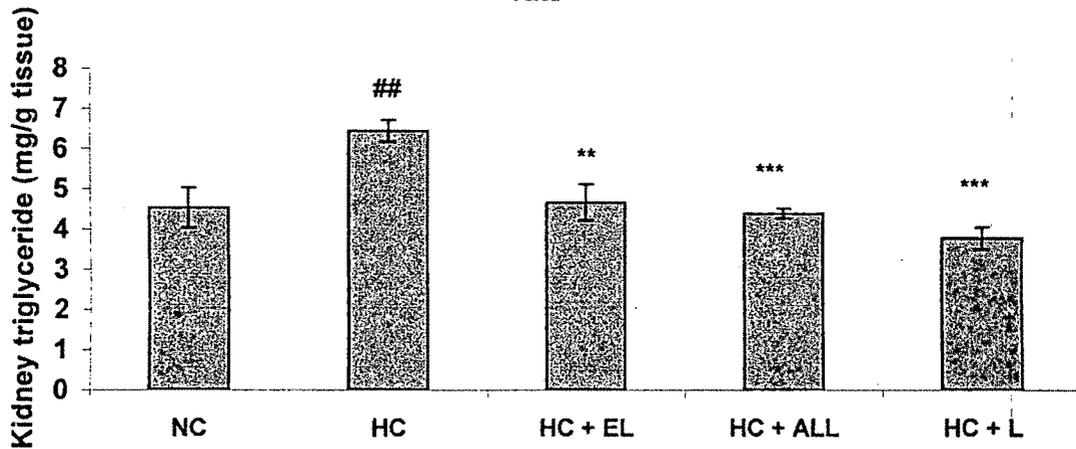
\*  $P < 0.05$ , \*\*  $P < 0.01$  as compared to HC at 6th week  
###  $P < 0.001$  as compared to NC at 6th week

**Fig 12: Effect of aqueous extracts of *E. littorale* (EL) and herbal combination (ALL) on hepatic triglyceride levels in cholesterol fed rats**



\*\*  $P < 0.01$ , \*\*\*  $P < 0.001$  as compared to HC at 6th week  
##  $P < 0.01$  as compared to NC at 6th week

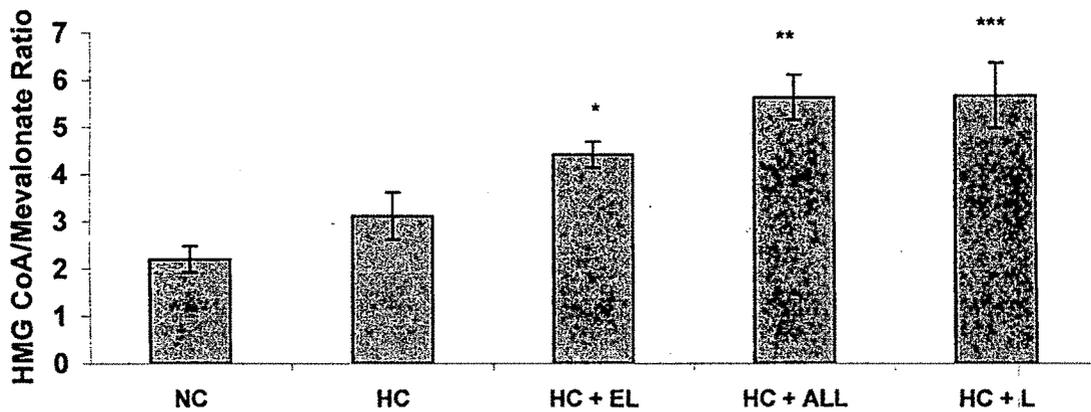
**Fig 13: Effect of aqueous extracts of *E. littorale* (EL) and herbal combination (ALL) on renal triglyceride levels in cholesterol fed rats**



\*\* P < 0.01, \*\*\* P < 0.001 as compared to HC at 6th week

## P < 0.01 as compared to NC at 6th week

**Fig 14: Effect of aqueous extracts of *E. littorale* (EL) and herbal combination (ALL) on hepatic HMG CoA reductase activity in cholesterol fed rats**



\* P < 0.05, \*\* P < 0.01, \*\*\* P < 0.001 as compared to HC

Increased Ratio = decreased HMG CoA reductase activity

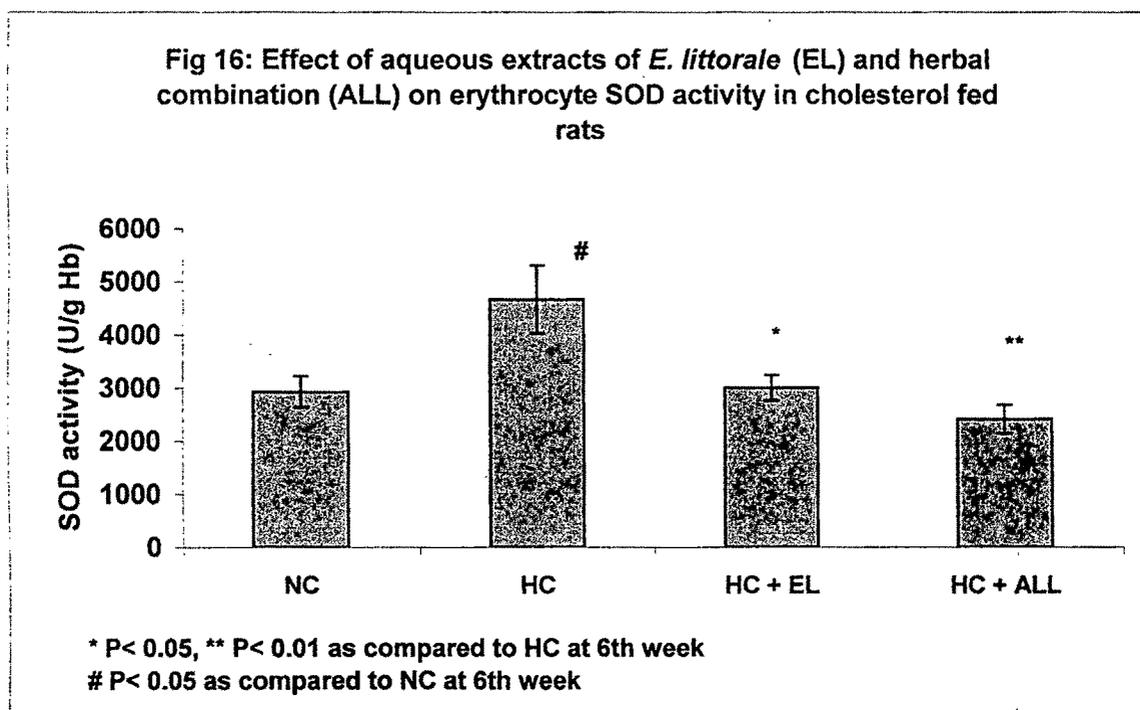
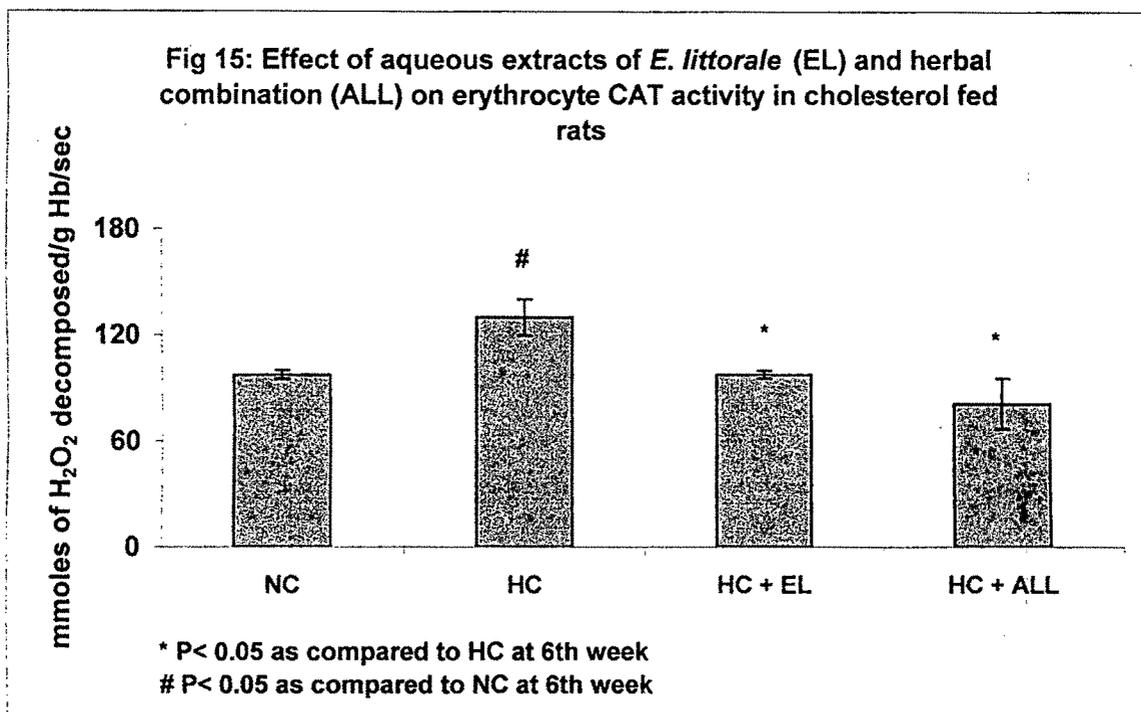
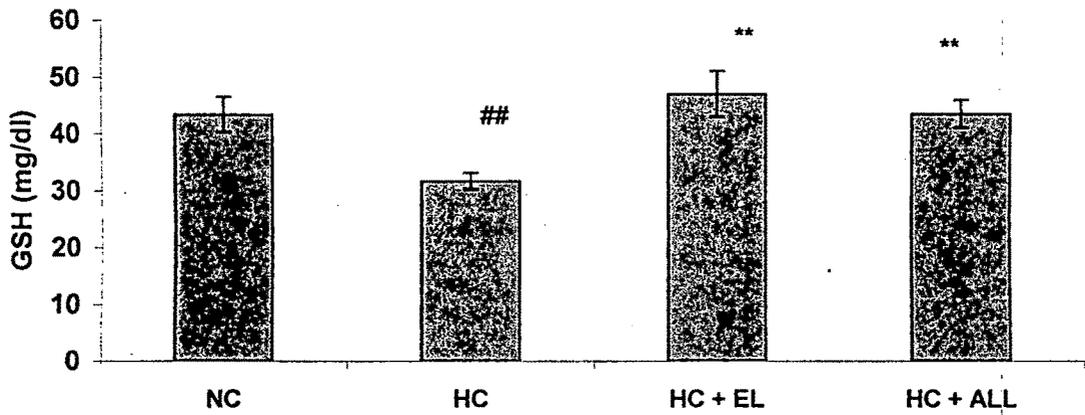
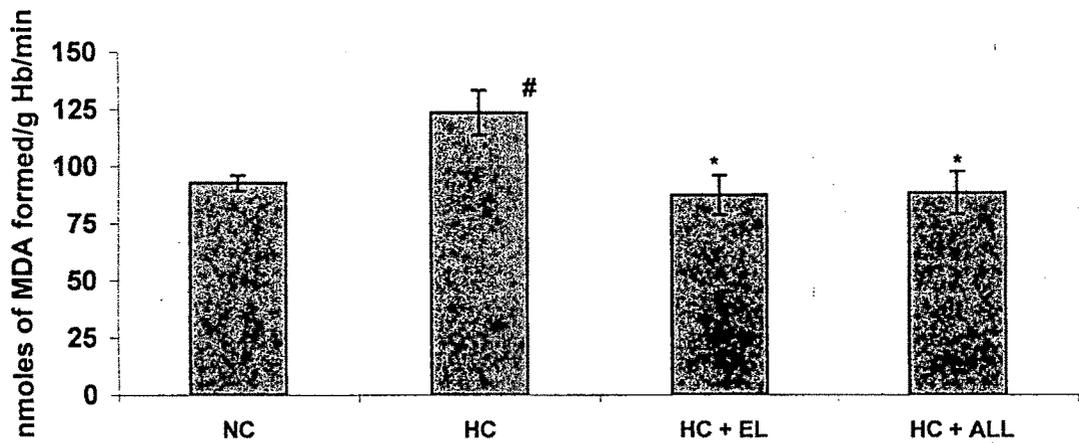


Fig 17: Effect of aqueous extracts of *E. littorale* (EL) and herbal combination (ALL) on blood GSH levels in cholesterol fed rats



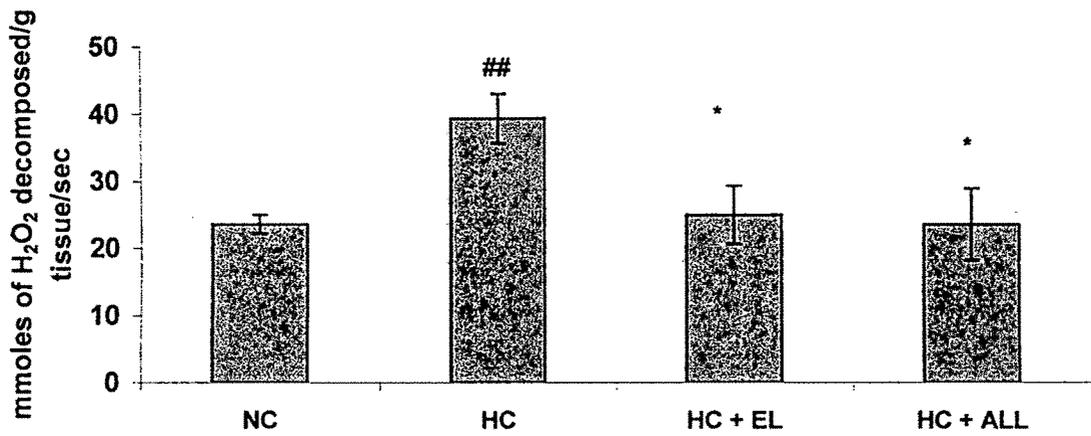
\*\* P < 0.01 as compared to HC at 6th week  
## P < 0.01 as compared to NC at 6th week

Fig 18: Effect of aqueous extracts of *E. littorale* (EL) and herbal combination (ALL) on erythrocyte LPO levels in cholesterol fed rats



\* P < 0.05 as compared to HC at 6th week  
# P < 0.05 as compared to NC at 6th week

Fig 19: Effect of aqueous extracts of *E. littorale* (EL) and herbal combination (ALL) on liver CAT activity in cholesterol fed rats



\* P < 0.05 as compared to HC at 6th week  
## P < 0.01 as compared to NC at 6th week

Fig 20: Effect of aqueous extracts of *E. littorale* (EL) and herbal combination (ALL) on kidney CAT activity in cholesterol fed rats

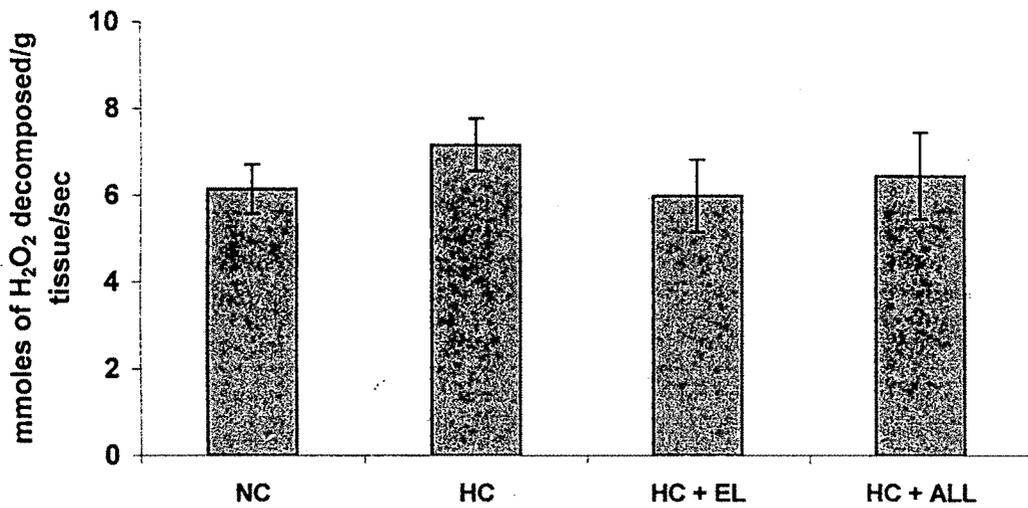
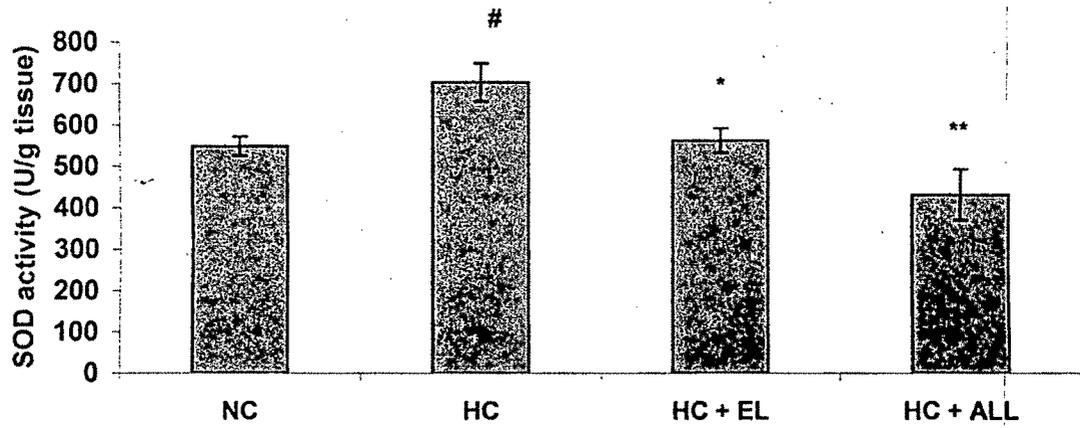


Fig 21: Effect of aqueous extracts of *E. littorale* (EL) and herbal combination (ALL) on liver SOD activity in cholesterol fed rats



\* P < 0.05, \*\* P < 0.01 as compared to HC at 6th week  
# P < 0.05 as compared to NC at 6th week

Fig 22: Effect of aqueous extracts of *E. littorale* (EL) and herbal combination (ALL) on kidney SOD activity in cholesterol fed rats

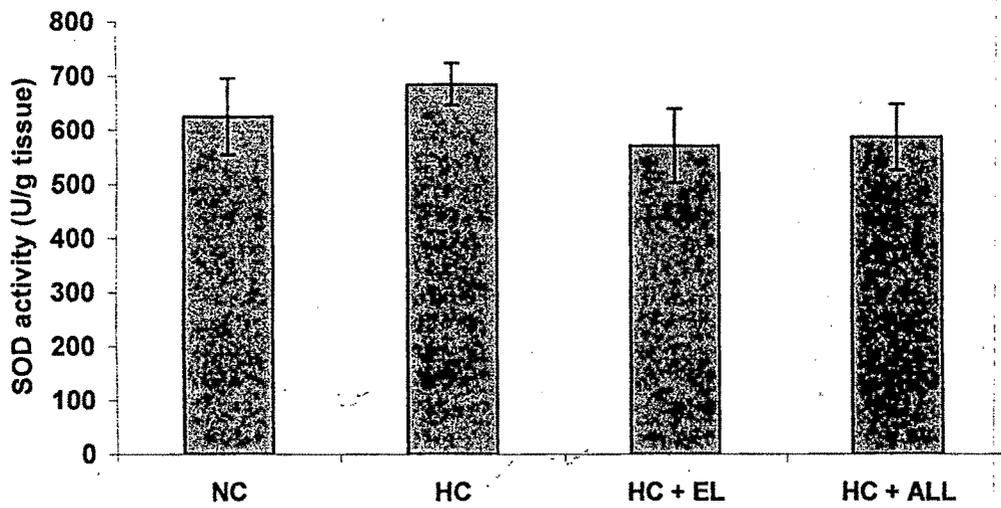
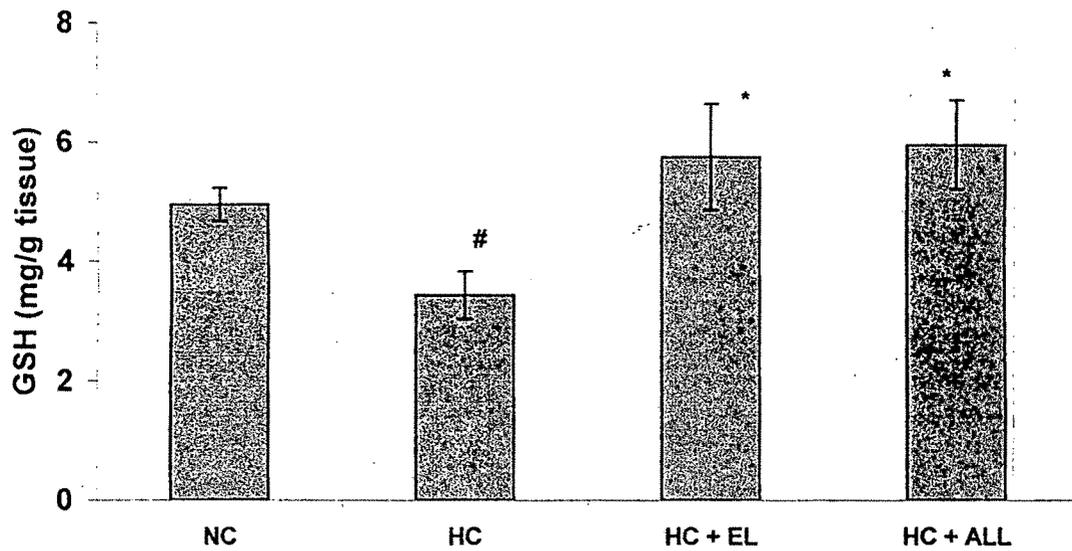
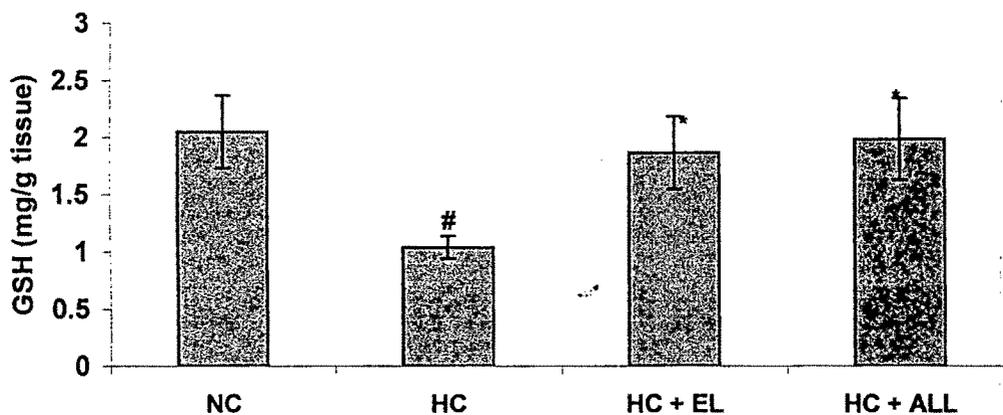


Fig 23: Effect of aqueous extracts of *E. littorale* (EL) and herbal combination (ALL) on liver GSH levels in cholesterol fed rats



\*  $P < 0.05$  as compared to HC at 6th week  
#  $P < 0.05$  as compared to NC at 6th week

Fig 24: Effect of aqueous extracts of *E. littorale* (EL) and herbal combination (ALL) on kidney GSH levels in cholesterol fed rats



\*  $P < 0.05$  as compared to HC at 6th week  
#  $P < 0.05$  as compared to NC at 6th week

