Chapter V

To study the antioxidant and hypolipidaemic effect of aqueous extract of herbal combination (*C. longa, E. officinalis, T. foenum-graecum, E. littorale*) in alloxan-induced diabetic rats

- Introduction
- Experimental design
- Results
- Discussion
- Summary

Introduction

Oxidative stress is an important trigger in the onset and progression of diabetes and its complications. There is emerging evidence that free radicals, eg. Reactive oxygen intermediates (ROI), reactive nitrogen species (RNS) and reactive chlorine species (RCS), make a significant contribution to the progression of diabetes and its complications (Tesfamariam, 1994). Autoxidation of glucose leads to the formation of ROI has already been shown (Wolff, 1993) and in type 2 diabetic patients, an elevated tension of redox stress within the islets of pancreas had been reported (Hayden and Tyagi, 2002). Imbalance of reactive oxygen species and antioxidants is one important pathogenic factor leading to insulin resistance through impaired stimulation of the insulin signaling pathway (Salonen et.al., 1997). Regeneration of glutathione is delayed in the presence of high glucose causing an impairment of the antioxidant defence (Kashiwagi et.al., 1994). Studies on glycation of proteins and Malliard reactions of glycated proteins have yielded indirect evidence for increased oxidative modifications of collagen in diabetes (Suzuki et.al., 1999).

Enhanced LDL oxidation is seen in diabetic patients both due to glucose-induced free radical production and insufficient antioxidant protection which promotes atherogenesis by formation of foam cells, modulating growth factor and cytokine expression (Bowie et.al., 1993). Abnormalities in lipoproteins are very common in both IDDM and NIDDM patients leading to obesity, hypertension and dyslipidemia. The increased VLDL, triglycerides, atherogenic small dense LDL cholesterol and the diminished amount of the anti-atherogenic, antioxidant, anti-inflammatory high density lipoprotein cholesterol would reduce the natural antioxidant reserve (Hayden and Tyagi,

2002). Hence an understanding of the pathophysiology and treatment of oxidative stress and dyslipidemia is essential for the successful management of diabetes and diabetic complications. Although biomedical science has designed therapeutic agents with a range of action to fight hyperglycemia, dyslipidemia and oxidative stress; the efficacy of these agents is compromised in several ways. Traditional medicinal systems, with a variety of herbal and non-herbal ingredients are thought to act on a variety of targets by various modes and mechanisms (Tiwari and Rao, 2002).

Traditional plant remedies have been used for centuries in the treatment of diabetes (Akhtar and Ali, 1984). As discussed earlier, *Curcuma longa, Emblica officinalis* and *Trigonella foenum-graecum* have been shown to have significant antioxidant (Kunchandy and Rao, 1990; Sharma, 1976; Sabu and Kuttan, 2002; Thirunavukkarasu et.al., 2003) and hypolipidaemic efficacy (Babu and Srinivasan, 1997; Manjunatha et.al., 2001; Brasch et.al., 2003). Methanolic extract administration of *Enicostemma littorale* to alloxan-induced diabetic rats significantly improved oxidant parameters by increasing reduced glutathione levels and decreased erythrocyte CAT activity and LPO levels as compared to untreated diabetic rats (Maroo et.al, 2003a) and the aqueous extract had showed hypolipidaemic effect in streptozotocin-induced diabetic rats (Murali et.al., 2002). In the present study, the antioxidant and hypolipidaemic efficacy of the combination of *Curcuma longa, Emblica officinalis, Trigonella foenum-graecum* and *Enicostemma littorale* was evaluated in alloxan-induced diabetic rats and were compared with the results obtained by administering aqueous extract on *E. littorale* alone. The free radical scavenging property of the herbal combination as well as *E. littorale in vitro* was also determined. Hypolipidaemic effect of *E. littorale* extract was

also seen in diabetic patients (as reported in chapter III of this thesis). Based on these reports, hypolipidaemic effect of aqueous extract of *E. littorale* alone was also evaluated in alloxan-induced diabetic rats.

Experimental Design

Experiments were carried out *in-vivo, in-vitro and* ex-*vivo*.

***In-vivo* experiments**

Hypolipidaemic and Antioxidant parameters

Hypolipidaemic and antioxidant effect of aqueous extract of *E. littorale* (EL) alone and herbal combination (ALL) was evaluated in alloxan-induced diabetic rats. Animals were divided into four major groups, consisting of untreated normoglycemic, treated normoglycemic, untreated diabetic and treated diabetic rats. Animals received aqueous extract (1.5g dry plant equivalent extarct/100g body weight/day) of herbal combination and another group received aqueous extract of *E. littorale* alone (1.5g dry plant equivalent extarct/100g body weight/day) for 20 days respectively. Blood samples were collected at 0^{th} and 20^{th} day. Lipid profile like serum total cholesterol, serum triglycerides and HDL cholesterol were estimated on 20^{th} day and VLDL and LDL values were calculated from the results. Antioxidant parameters such as erythrocyte CAT, SOD and GPx activity, LPO and blood GSH levels were estimated on 0^{th} and 20^{th} day. Tissue (liver and kidney) CAT, SOD and GPx activity and LPO and GSH levels were estimated on 20^{th} day after sacrificing the animal.

***In-vitro* experiment**

In vitro experiments were performed to monitor the free radical (DPPH free radical, superoxide radical, hydroxyl radical, nitric oxide radical) scavenging effect of the

different concentrations of aqueous extracts of herbal combination and *E. littorale* by using generating system as described in chapter II. Vitamin C was used as reference.

***Ex-vivo* experiment**

Ex-vivo experiments were performed in liver homogenate to see the effect of herbal combination and *E. littorale* aqueous extract after LPO induction and protection against GSH oxidation. Experiments were also done with isolated rat pancreatic islets by incubating the herbal combination aqueous extract as described in chapter II to monitor the antioxidant effect of the extract. Rat pancreatic islets were isolated as described in chapter II. These islets were picked up manually in a batch of 10 islets under stereomicroscope in each tube for all the experiments. Islets were incubated with alloxan and different concentrations of herbal combination aqueous extract and Nitric Oxide scavenging in the media released, GSH and LPO levels of the isolated rat pancreatic islets were determined. Vitamin C was used as reference.

Results

***In-vivo* experiments**

(i) Hypolipidaemic parameters

Diabetes causes dyslipidemia which was clearly shown by the alloxan-induced diabetic rats with increased serum cholesterol levels, serum triglycerides, LDL cholesterol, VLDL cholesterol and decreased levels of HDL cholesterol. Aqueous extracts of *E. littorale* and herbal combination treated normoglycaemic rats at 20^{th} day showed significant decrease in serum total cholesterol with 16% ($P<0.05$) and 33.5% ($P<0.01$) and a decrease of 40.5% ($P<0.05$) and 43.2% ($P<0.01$) in serum LDL cholesterol levels respectively, as compared to values of untreated normoglycaemic rats

at 20th day (Fig 1, 2). Both extracts in diabetic rats on 20th day showed significant amelioration in lipid profile as compared to untreated diabetic rats. Herbal combination showed a decrease of 29.3%, 43.9%, 43.8% and 48.6% in serum cholesterol, serum triglycerides, LDL cholesterol and VLDL cholesterol and an increase of 39.3% in HDL cholesterol levels in treated diabetic rats, whereas the *E. littorale* treatment showed a decrease of 20.2%, 25%, 25% and 32.6% in serum cholesterol, serum triglycerides, LDL cholesterol and VLDL cholesterol and an increase of 32.8% in HDL cholesterol levels respectively (Fig 3, 4)

(Ii) Antioxidant parameters

In treated normoglycaemic rats, there was significant decrease in erythrocyte CAT and SOD activity in both groups as compared to 20th day value of untreated normoglycaemic rats. Erythrocyte SOD activity was significantly decreased and blood GSH levels increased in extract treated normoglycaemic rats on 20th day as compared to 0th day value. The other antioxidant parameters of normoglycaemic rats remained unchanged (Fig 5 – 9).

Alloxan treatment caused decrease in blood reduced glutathione (GSH) levels with increase in erythrocyte catalase (CAT) activity, superoxide Dismutase (SOD) activity, glutathione peroxidase (GPx) and lipid peroxidation (LPO) levels in diabetic rats. The single dose administration of herbal combination (1.5g dry plant equivalent extarct/100g body weight/day) when compared to *E. littorale* (1.5g dry plant equivalent extarct/100g body weight/day) (Maroo et.al., 2002) at 20th day, showed a significant decrease in erythrocyte CAT, SOD, GPx, and LPO levels and a significant increase in GSH levels as compared to 0th day value and also with 20th day values of untreated

diabetic rats. Comparatively, herbal combination showed more antioxidant potential with a decrease of 44.7%, 47.5%, 32.6% and 46.9% in erythrocyte CAT, SOD, GPx activities and LPO levels respectively and an increase of 25.1% in blood GSH levels at 20th day as compared to values at 0th day of treated diabetic rats. Whereas, *E. littorale* extract treatment showed a decrease of 35.6%, 32%, 27.5% and 37.7% in erythrocyte CAT, SOD, GPx activities and LPO levels respectively and an increase of 23% in blood GSH levels (Fig 10 – 14).

There was a significant decrease in hepatic and renal CAT, SOD, GPx and LPO levels in both herbal combination and *E. littorale* treated groups and an increase in GSH levels, which were impaired in the untreated diabetic rats. When compared with tissue antioxidant parameters also, herbal combination showed more effect than *E. littorale* in the antioxidant parameters studied (Fig 15 – 24)

***In-vitro* experiments**

To understand the antioxidant mechanism of action the following experiments were carried out. Aqueous extract of *E. littorale* showed DPPH free radical scavenging activity with IC_{50} at 4 mg dry plant equivalent weight of extract, whereas herbal combination showed DPPH free radical scavenging at an IC_{50} of approximately 2 mg dry weight of extract (Fig 25). The amount of *E. littorale* aqueous extract dry plant equivalent weight needed for 50% inhibition (IC_{50}) of hydroxyl radical and nitric oxide radical scavenging were 247.32 µg/ml and 320.54 µg/ml respectively but superoxide scavenging effect was not detected, whereas herbal combination showed 50% inhibition (IC_{50}) of superoxide radical, hydroxyl radical and nitric oxide radical scavenging at 72.45 µg/ml, 98.68 µg/ml, 122.43 µg/ml respectively (Table I)

***Ex-vivo* experiment**

The antioxidant activity of aqueous extracts of *E. littorale* and herbal combination was essayed in rat liver homogenates by inducing lipid peroxidation using 0.5 mM $FeSO_4$. Both extracts showed the reduction in lipid peroxidation induced by ferrous sulphate. The degree of depletion was more with the herbal combination (ED_{50} 150 μg) as compared to *E. littorale* (ED_{50} 200 μg) (Table II). It is generally seen that in normal condition GSH content decreases after 20 minutes due to auto-oxidation and hence the experiment was conducted incubating the extracts to determine the antioxidant effect. In both the extract treated groups rate of GSH oxidation was reduced (Fig 26).

The antioxidant effect of *E. littorale* and herbal combination were evaluated in the isolated rat pancreatic islets. Islets were incubated with or without 0.5 mM alloxan (normally used concentration range is 0.1 – 1.0 mM to induce oxidative stress in the islets) along with 5 and 20 μg of the extracts (this range of 5 – 20 μg was used in the insulin release experiments as reported by Maroo et.al., 2002) as described in chapter II. The alloxan treated group showed an increase in LPO and a decrease in GSH levels as compared to control group. The EL-5 and EL-20 when preincubated with alloxan showed a decrease in LPO which was significant ($P < 0.05$) in the EL-20 + alloxan as compared to the alloxan group. Also, the group where alloxan was first incubated and then EL-5 and EL-20 was added, LPO decrease was not significant. (Fig 27). Similarly, the EL-20 + alloxan (pre-incubated) and alloxan + EL-20 groups showed a significant increase in the GSH levels as compared to the alloxan treated group ($P < 0.05$. But incubating with herbal combination (ALL), there was significant decrease in LPO and protective effect against GSH oxidation in all the groups (Fig 28).

Islets showed an increase in NO production in alloxan treated group as compared to the control. Preincubation with EL-5 and EL-20 with alloxan showed a significant decrease in NO production in EL-20 group (EL-20 + alloxan) from islets. But preincubation with alloxan and then EL-5 and EL-20 (Alloxan + EL-5; Alloxan + EL-20) didn't cause any significant decrease. ALL-5 + alloxan didn't show any significant change, but ALL-20 + alloxan and Alloxan + ALL-20 showed significant decrease in alloxan-induced NO production from isolated pancreatic islets (Fig 29).

Discussion

Abnormalities in lipoproteins are very common in both individuals with non-insulin-dependent diabetes (NIDDM) and insulin-dependent diabetes (IDDM). Diabetes and coronary heart disease share many of the same risk factors, such as disorders of lipid metabolism and hypertension. Hyperglycemia induces a large number of alterations at the cellular level of vascular tissue and thus potentially accelerate the atherosclerotic process. Hence, control of dyslipidemia, a complication of diabetes must be severely dealt with. Most of the plants that show antidiabetic effect are reported to show hypolipidaemic effect as well. Administration of curcumin, from *Curcuma longa* to streptozotocin-induced diabetic rats decreased serum cholesterol, triglycerides and phospholipids which were elevated in untreated diabetic rats (Babu ans Srinivasan, 1997). Defatted fenugreek seed powder was given to IDDM patients at a dose of 100 grams daily in two divided doses over a 10-day period. The fenugreek-treated IDDM patients exhibited a 54% decrease in 24-hour urinary excretion of glucose, as well as a reduction in total cholesterol, LDL, VLDL, and triglycerides (Sharma et.al., 1990). Animal studies have also demonstrated the hypoglycemic and hypolipidemic effects of fenugreek (Khosla

et.al., 1995; Petit et.al., 1995). Evaluation of *Emblica officinalis* (Amla) fresh juice in cholesterol-fed rabbits lowered serum cholesterol, TG, phospholipid and LDL levels by 82%, 66%, 77% and 90%, respectively. Similarly, the tissue lipid levels also showed a significant reduction and aortic plaques were regressed in *E. officinalis* juice treated rabbits (Mathur et.al., 1996). In the present study, aqueous extracts of herbal combination and *E. littorale* showed significant decrease in serum cholesterol levels, serum triglycerides, LDL cholesterol, VLDL cholesterol and increased levels of HDL cholesterol in treated diabetic rats as compared to 20^{th} day values of untreated diabetic rats. The effect was more seen in the herbal combination probably because fenugreek, emblica and turmeric, the components of the extract are known potent hypolipidaemic agents. Thus the combination had demonstrated increased efficacy.

Oxidative stress can be described as an imbalance between the production and activity of reactive oxygen species and the defence mechanism. Oxidative stress is implicated in the etiopathogenesis of a variety of human diseases due to various free radicals like – hydroxyl radicals, superoxide radicals, nitric oxide radicals, peroxy radicals. (Beck and Levander, 1998). Excess oxidative stress has captured considerable attention as a potential mechanism for the increased vascular disease in diabetics. Several synthetic and natural antioxidants have shown to decrease the toxicity by oxidative stress Search for plants has lead to the identification of various herbs, which could be used as antioxidants.

Curcuma longa, Emblica officinalis, Trigonella foenum-graecum and *Enicostemma littorale* were selected in the presented study as they showed good antidiabetic properties individually. When these plants were given in combination as

aqueous extract to alloxan-induced diabetic rats and compared with the results of diabetic rats, where *E. littorale* aqueous extract was given alone, it was seen that there was significant amelioration in erythrocyte CAT, SOD, GPx activities and increased blood GSH levels along with decrease in erythrocyte LPO levels. The effects were same in the case of tissue antioxidants as well, but comparatively it was observed that the herbal combination was more potent as an antioxidant than *E. littorale* alone. By combining the selected medicinal plants, the efficacy of the antioxidant potential was increased. *C. longa* contains diarylheptanoids, curcumin, demethoxycurcumin and bisdemethoxycurcumin which are good free radical scavengers (Song e.al., 2001) and curcumin had been reported to inhibit lipid peroxidation and maintained the activities of antioxidant enzymes such as – superoxide dismutase, catalase, glutathione peroxidase (Pulla Reddy & Lokesh, 1992). *Emblica* consisting of emblicanin A (37%), emblicanin B (33%), punigluconin (12%) and pedunculagin (14%) showed significant modulation of antioxidant enzymes and decreased lipid peroxidation levels in rat brain (Bhattacharya et.al., 1999). Fenugreek seeds has also showed normalization of disrupted free radical metabolism in diabetic animals its supplementation in the diet by decreasing lipid peroxidation and increasing antioxidant status (Ravikumar & Anuradha, 1999). The antioxidant effect of a methanolic extract of *E. littorale* was reported earlier by our lab (Maroo et.al., 2003b). Hence, the combining of the above plants showed an increased antioxidant effect as compared to *E. littorale* alone taken as reference.

To understand the antioxidant mechanism of action, *invitro* studies were carried out. It was seen that *E. littorale* showed DPPH free radical, hydroxyl radical and nitric oxide radical scavenging property, whereas herbal combination showed DPPH free

radical, hydroxyl radical, superoxide radical and hydroxyl radical scavenging properties and the ED_{50} was much decreased than the former, again projecting the increased efficacy of the herbal combination. The free radical scavenging effect of extracts seems to be one of the reason, that there is a decreased activity of antioxidant enzymes in treated diabetics as compared to untreated diabetic rats.

Exvivo experiments using liver homogenates were carried out inducing LPO, it was seen that both *E. littorale* and herbal combination extracts exhibited protection against induced LPO when incubated with the same. Moreover the extracts were able to protect auto-oxidation of GSH content of liver homogenate. The degree of depletion of induced LPO and protection against GSH auto-oxidation was more with the combination extract, again confirming its efficacy due to it's various properties mentioned above. Moreover, reports have shown that the antioxidant status of the islets is weak, the mRNA, protein and enzyme activity of hydrogen peroxide inactivating enzymes CAT and GSH-Px in rat pancreatic islets is extremely low as compared to that of the liver (Tiedge et.al., 1997). The antioxidant effect of aqueous extracts of *E. littorale* and herbal combination evaluated in the isolated rat pancreatic islets incubated with alloxan showed a decreased LPO levels and NO production, and protected against GSH oxidation, mainly in those groups were the extract were pre-incubated. The concentration used were 5 and 20 μg of dry plant equivalent and *E. littorale* showed good efficacy in 20 μg, where as the herbal combination was effective in both 5 and 20 μg concentrations.

Thus, the above results shows that combining the selected medicinal plants was able to increase the antioxidant and hypolipidaemic efficacy, as compared to the single *E. littorale* aqueous extract. As diabetes mellitus is a complicated syndrome, combination

therapy might be an effective means of answering some of its serious outcomes and thus a potential candidate for therapeutic purposes.

Summary

Both aqueous extracts of *E. littorale* and herbal combination showed significant decrease in serum cholesterol, triglycerides, VLDL and LDL cholesterol levels and a significant increase in HDL cholesterol levels, with the efficacy seen more in combination group.

Similarly, both demonstrated good antioxidant efficacy and the extract treatment decreased CAT, SOD, GPx activities, LPO levels and increased GSH levels in erythrocyte and tissue (liver & kidney) as compared to untreated diabetic rats value. The efficacy was seen more in herbal combination treated rats.

E. littorale aqueous extract showed DPPH free radical, hydroxyl radical and nitric oxide radical scavenging property, whereas herbal combination showed DPPH free radical, hydroxyl radical, superoxide radical and hydroxyl radical scavenging properties and the ED_{50} was much decreased than the former.

E. littorale and herbal combination extracts exhibited protection against induced LPO and were able to protect auto-oxidation of GSH content of liver homogenate. The degree of depletion of induced LPO and protection against GSH auto-oxidation was more with the combination extract.

E. littorale (20 μg) showed antioxidant potential when it was preincubated with isolated pancreatic islets inhibiting LPO and NO production and GSH oxidation induced

by alloxan, where as herbal combination showed better efficacy than *E. littorale* in both 5 and 20 μg concentrations.

Herbal combination (*Curcuma longa, Emblica officinalis, Trigonella foenum-graecum* and *Enicostemma littorale*) is more potent as a hypolipidaemic and antioxidant agent, when compared to *E. littorale* alone.

Table I: *In vitro* antioxidant activity of herbal combination and *E. littorale* aqueous extract

Aqueous Extract	Amount needed for 50% inhibition (µg/ml)		
	Superoxide radical generation	Hydroxyl radical generation	Nitric oxide radical generation
E. littorale	ND	247.32 ± 12.03	320.54 ± 17.22
Herbal combination	72.45 ± 4.09	98.68 ± 2.56	122.43.± 8.93
Vitamin C	18.12 ± 0.04	42.10 ± 2.30	24.65 ± 4.30

Values are expressed as mean ± SE (n = 4 – 5)

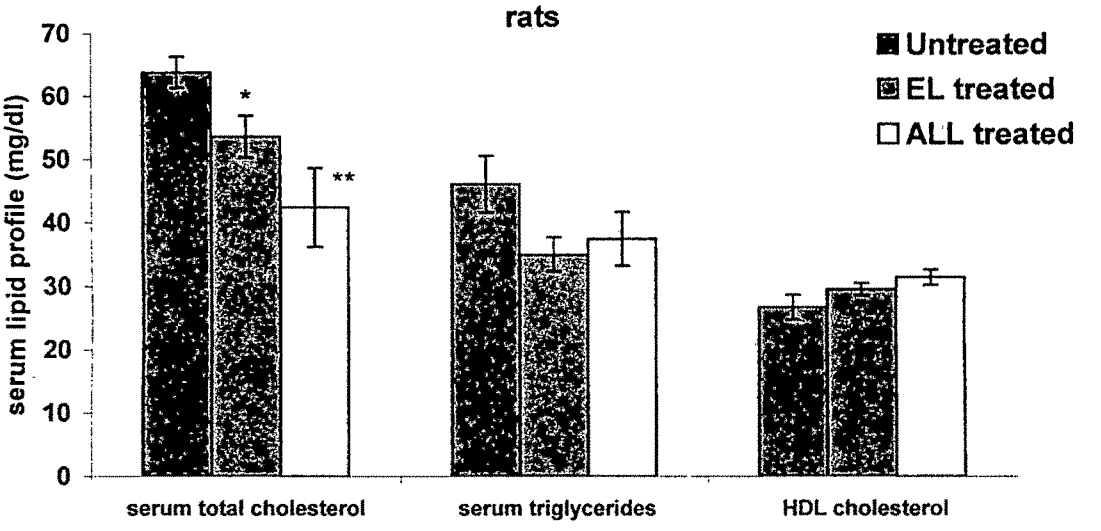
Table II: Inhibition of induced lipid peroxidation (LPO) by *E. littorale* and herbal combination aqueous extracts

Dose (µg/ml)	*E. littorale* Aqueous extract	Herbal combination aqueous extract
0	480.65 ± 18.76	
50	400.01 ± 14.32 *	377.02 ± 9.78 *
150	366.01 ± 15.33 *	222.34 ± 10.32 *
200	247. 32 ± 14.55 *	143.24 ± 17.22 *
250	200. 01 ± 11.10 *	110.99 ± 9.87 *
ED_{50}	200 µg/ml	150 µg/ml

Values expressed as nmol MDA formed / 100 mg protein (n = 6)

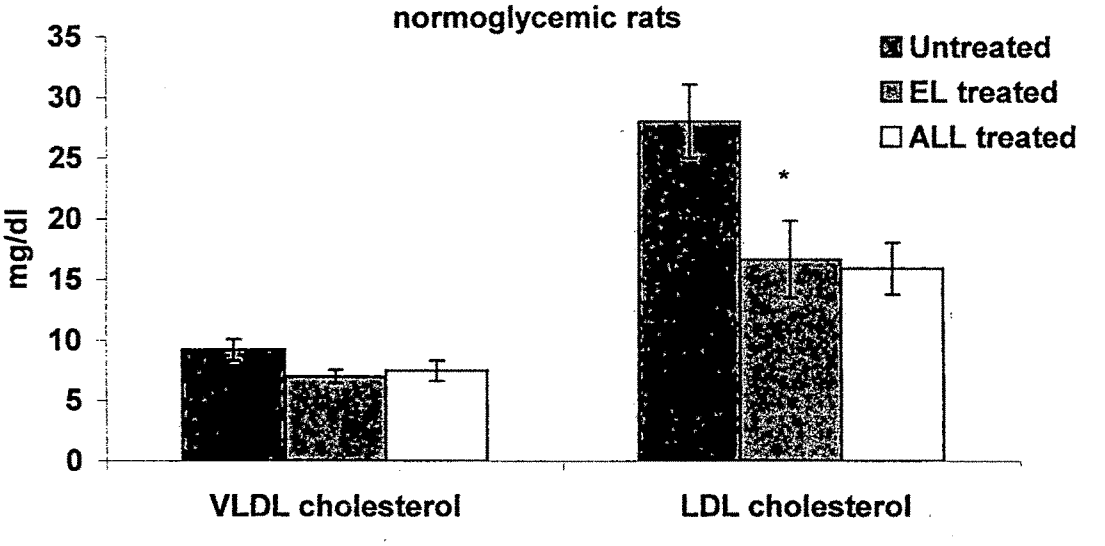
* $P < 0.001$

Fig 1: Effect of aqueous extract of *E. littorale* and herbal combination (ALL) on serum lipid profile in normoglycemic rats



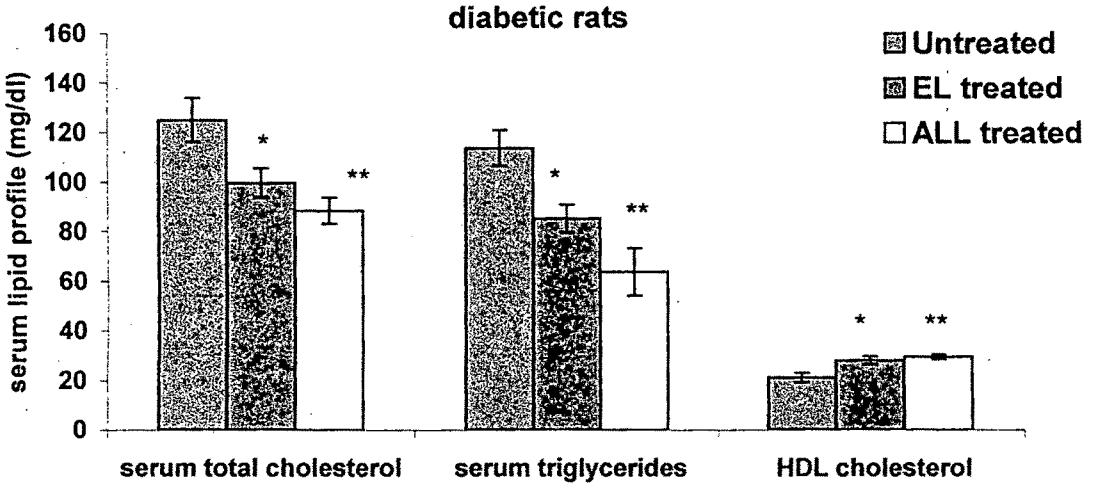
* P< 0.05, ** P< 0.01 as compared to untreated rats

Fig 2: Effect of aqueous extract of *E. littorale* and herbal combination (ALL) on serum VLDL and LDL levels in normoglycemic rats



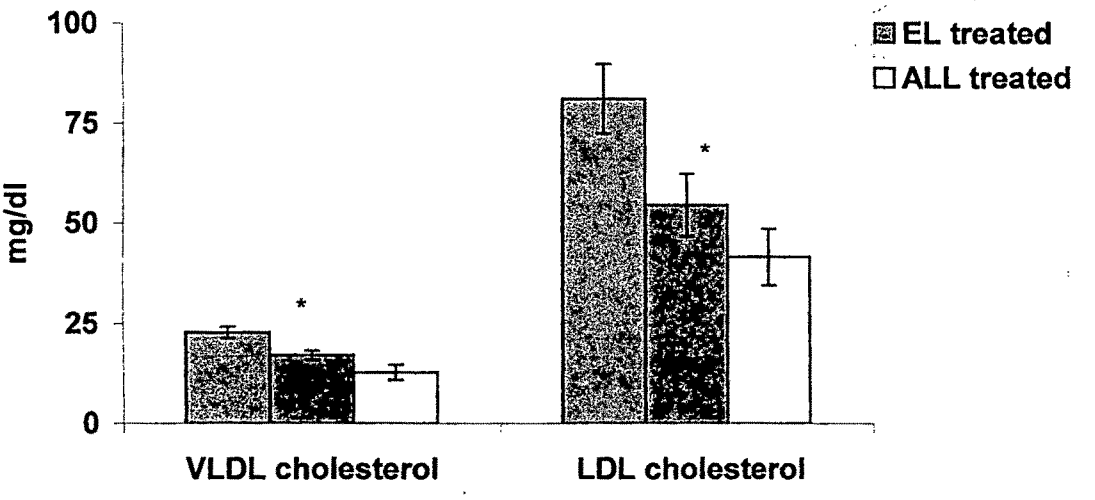
* P< 0.05, ** P< 0.01 as compared to untreated rats

Fig 3: Effect of aqueous extract of *E. littorale* and herbal combination (ALL) on serum lipid profile in alloxan-induced diabetic rats



* P< 0.05, ** P< 0.01 as compared to untreated rats

Fig 4: Effect of aqueous extract of *E. littorale* and herbal combination (ALL) on serum VLDL and LDL levels in alloxan-induced diabetic rats



* P< 0.05, ** P< 0.01 as compared to untreated rats

Fig 5: Effect of aqueous extract of *E. littorale* and herbal combination (ALL) on erythrocyte CAT activity of normoglycemic rats on 0th and 20th day

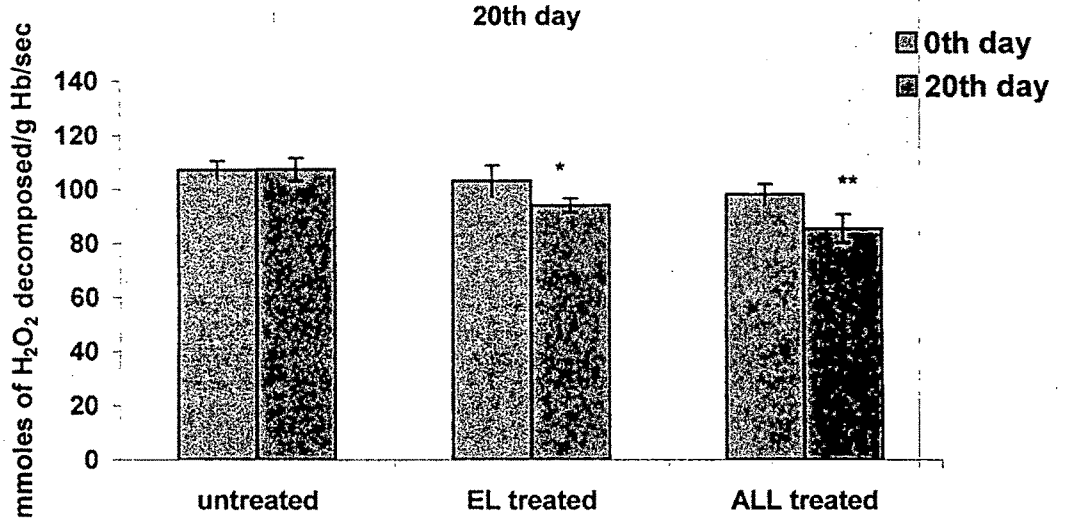
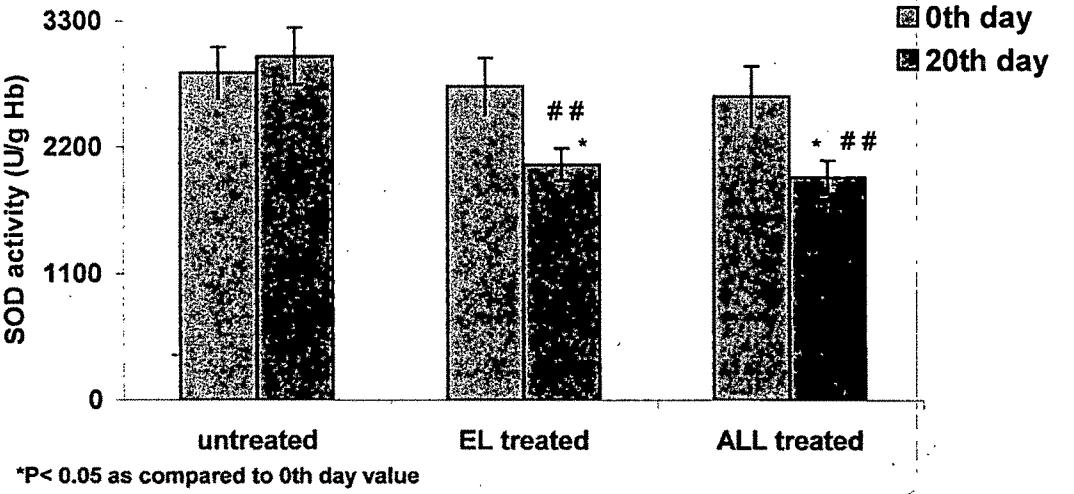


Fig 6: Effect of aqueous extract of *E. littorale* and herbal combination (ALL) on erythrocyte SOD activity of normoglycemic rats on 0th and 20th day



*P< 0.05 as compared to 0th day value
P<0.01 as compared to 20th day value of untreated rat

Fig 7: Effect of aqueous extract of *E. littorale* and herbal combination (ALL) on erythrocyte GPx activity of normoglycemic rats on 0th and 20th day

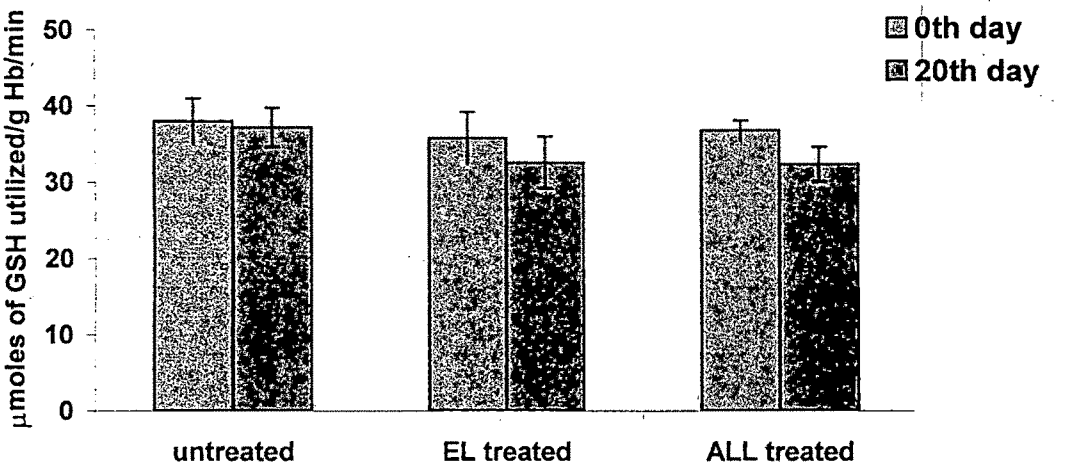


Fig 8: Effect of aqueous extract of *E. littorale* and herbal combination (ALL) on erythrocyte LPO levels of normoglycemic rats on 0th and 20th day

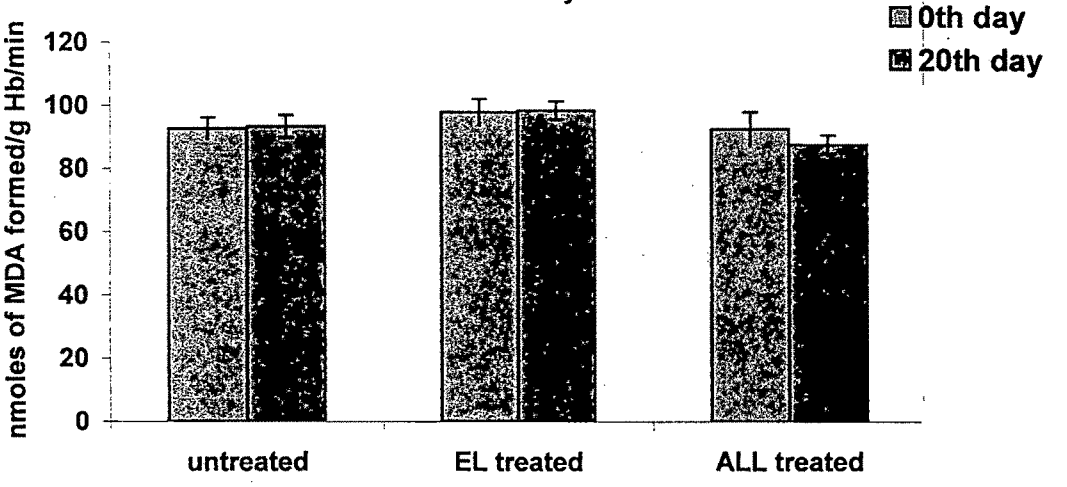


Fig 9: Effect of aqueous extract of *E. littorale* and herbal combination (ALL) on whole blood GSH levels of normoglycemic rats on 0th and 20th day

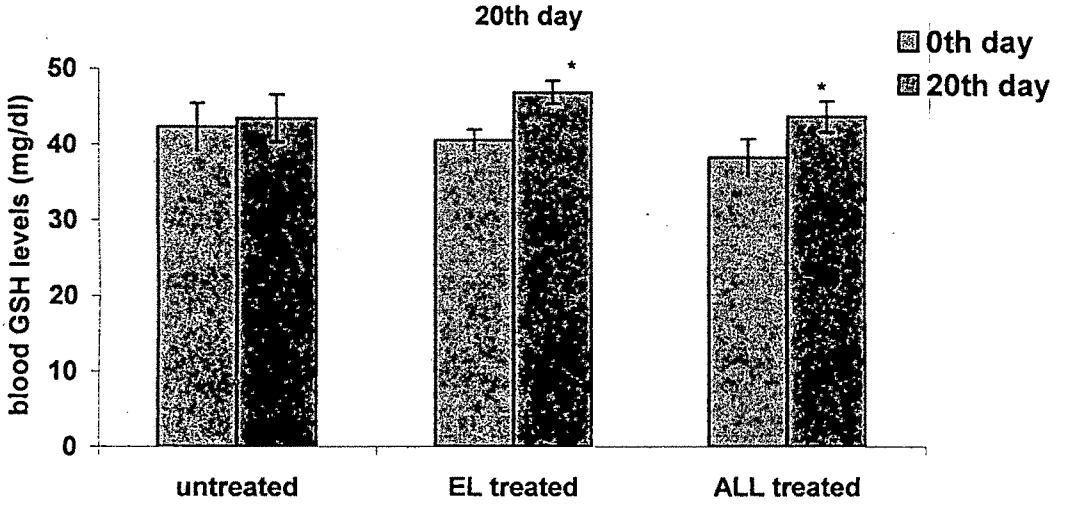


Fig 10: Effect of aqueous extract of *E. littorale* and herbal combination (ALL) on erythrocyte CAT activity of normoglycemic rats on 0th and 20th day

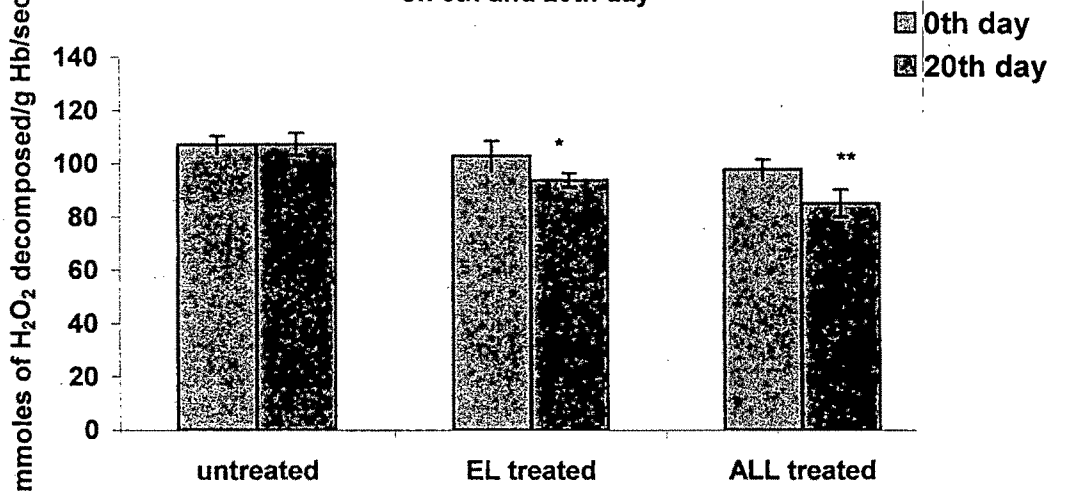
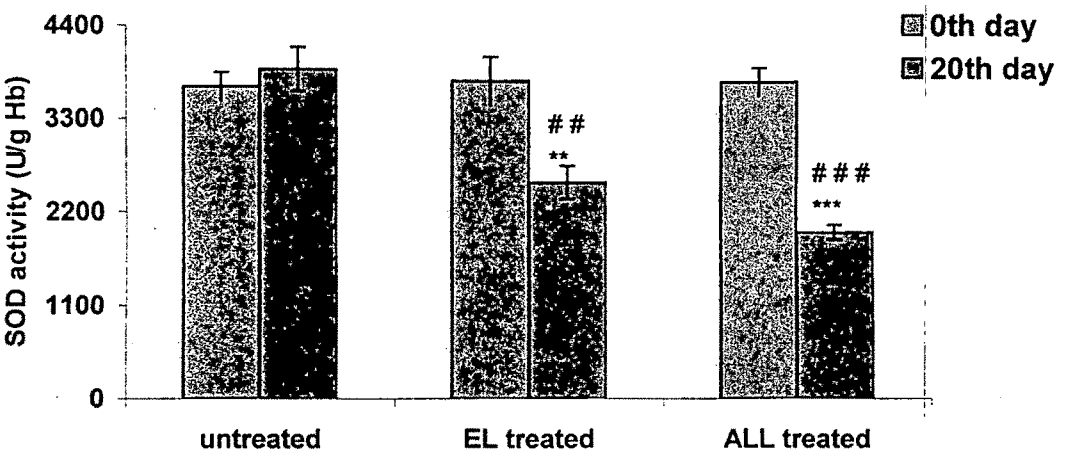


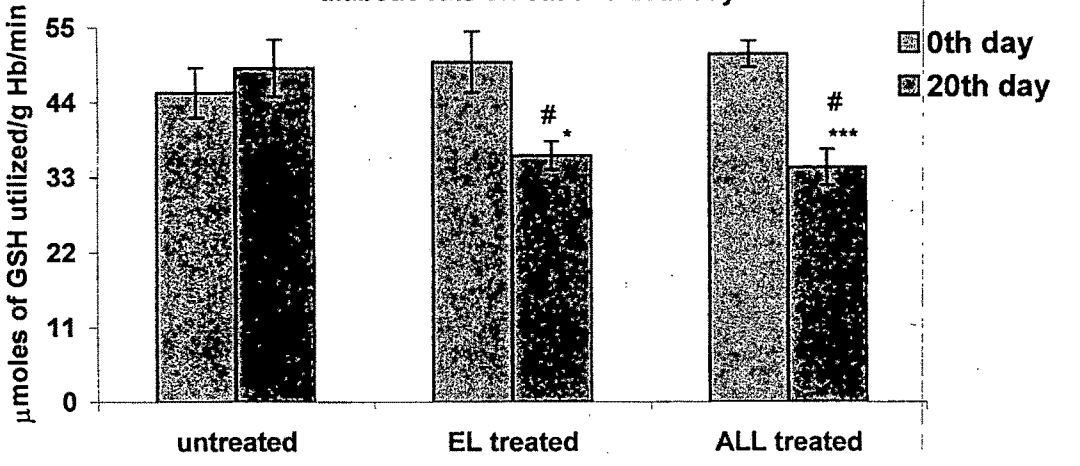
Fig 11: Effect of aqueous extract of *E. littorale* and herbal combination (ALL) on erythrocyte SOD activity of alloxan-induced diabetic rats on 0th and 20th day



** P< 0.01, *** P< 0.001 as compared to 0th day value

P<0.01, ### P< 0.001 as compared to 20th day value of untreated rat

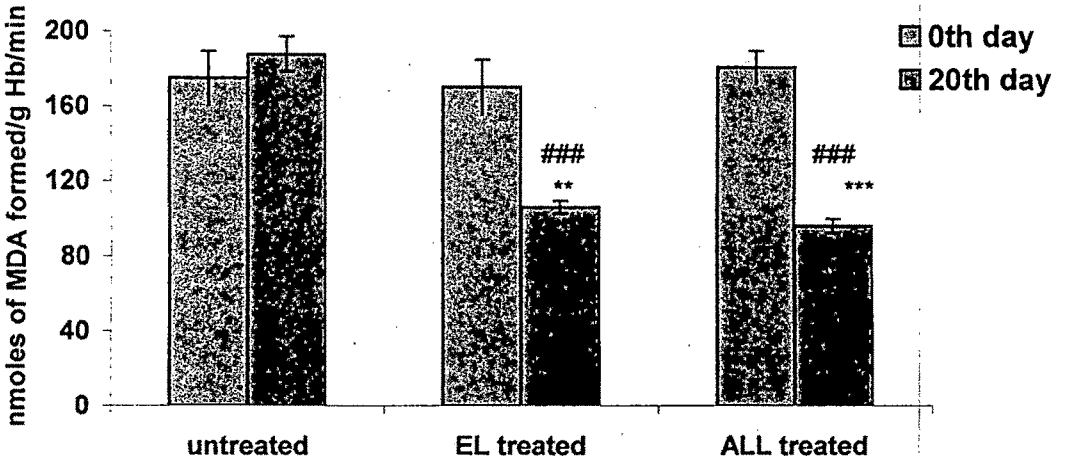
Fig 12: Effect of aqueous extract of *E. littorale* and herbal combination (ALL) on erythrocyte GPx activity of alloxan-induced diabetic rats on 0th and 20th day



* P< 0.05, *** P< 0.001 as compared to 0th day value

# P<0.05 as compared to 20th day value of untreated rat

Fig 13: Effect of aqueous extract of *E. littorale* and herbal combination (ALL) on erythrocyte LPO levels of alloxan-induced diabetic rats on 0th and 20th day



** P< 0.01, *** P< 0.001 as compared to 0th day value
P< 0.001 as compared to 20th day value of untreated rat

Fig 14: Effect of aqueous extract of *E. littorale* and herbal combination (ALL) on whole blood GSH levels of alloxan-induced diabetic rats on 0th and 20th day

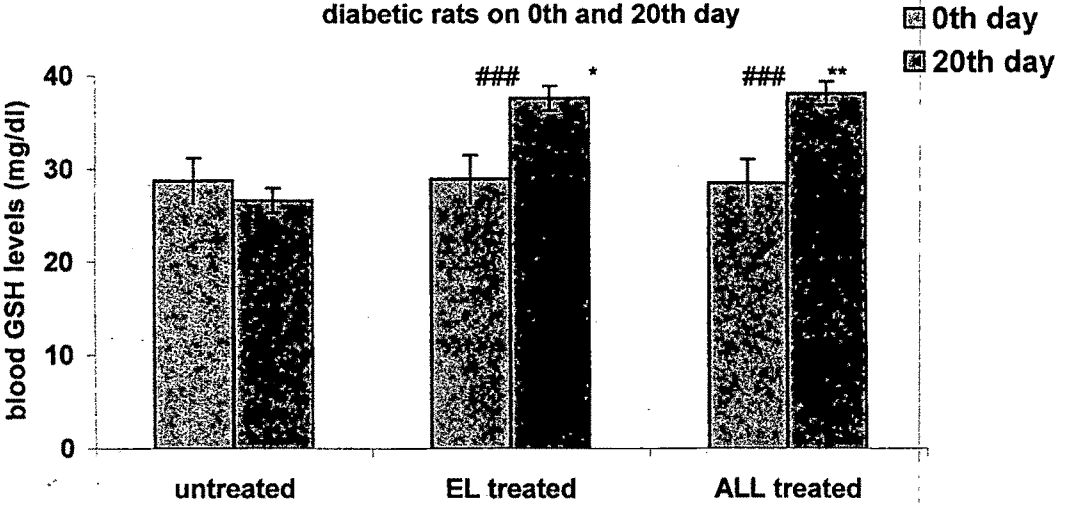
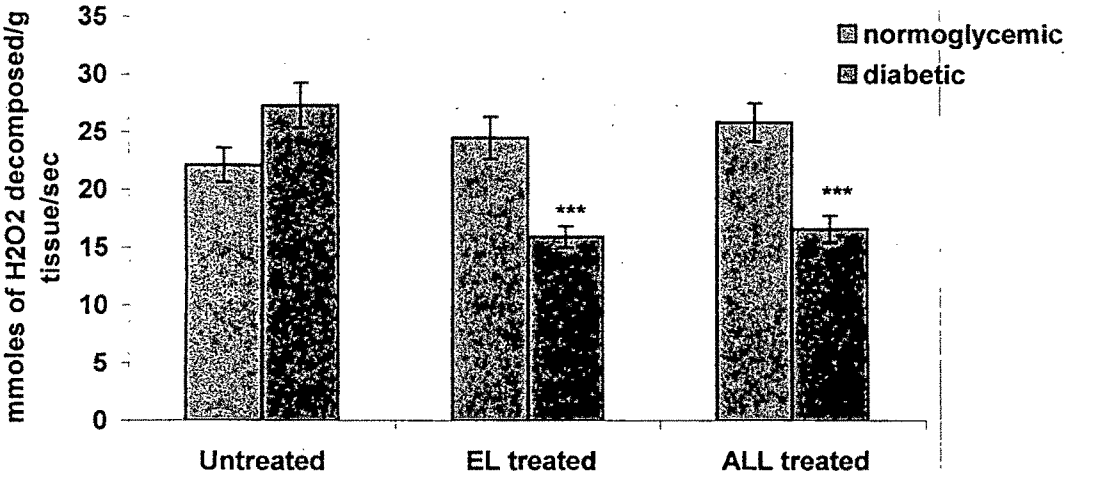
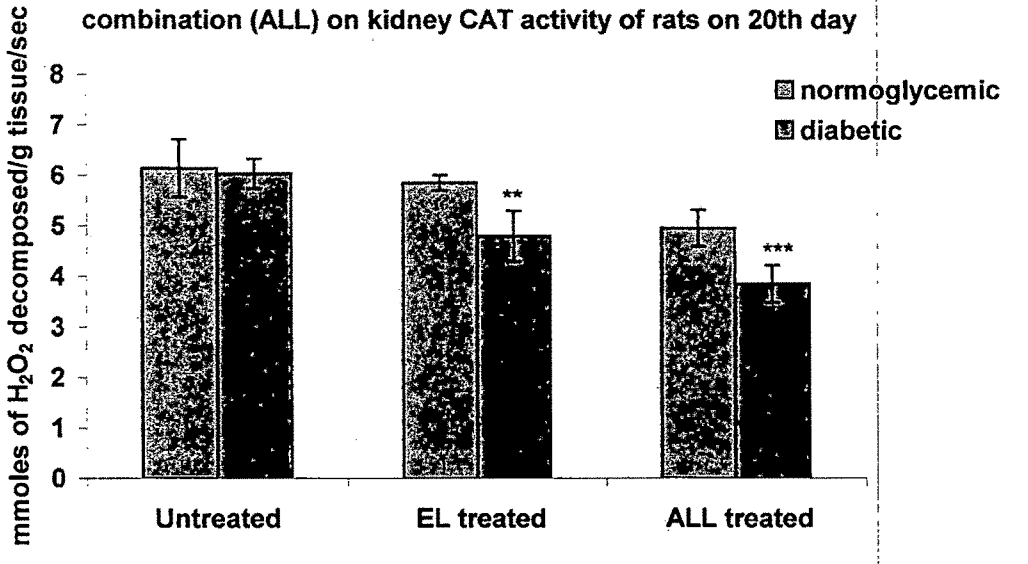


Fig 15: Effect of aqueous extract of *E. littorale* and herbal combination (ALL) on liver CAT activity of rats on 20th day



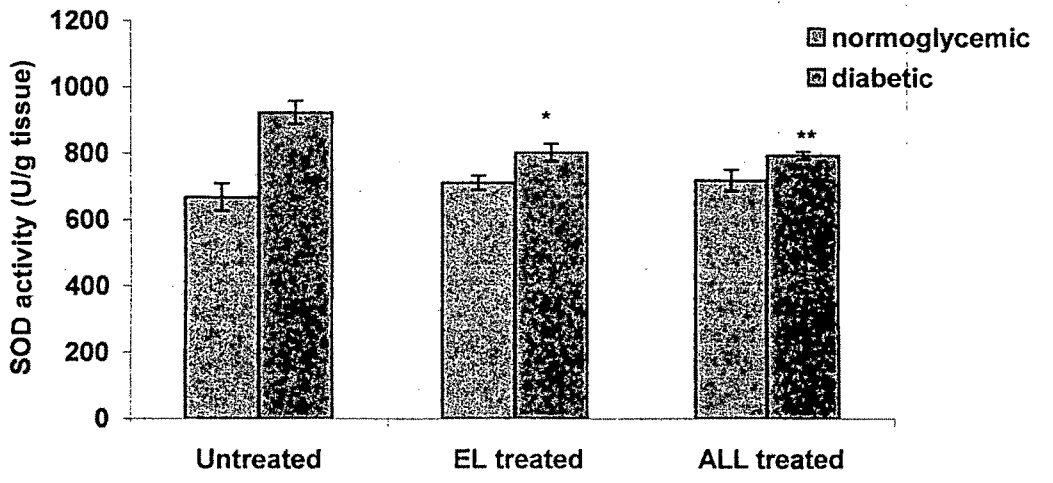
*** P< 0.001 as compared to untreated diabetic

Fig 16: Effect of aqueous extract of *E. littorale* and herbal combination (ALL) on kidney CAT activity of rats on 20th day



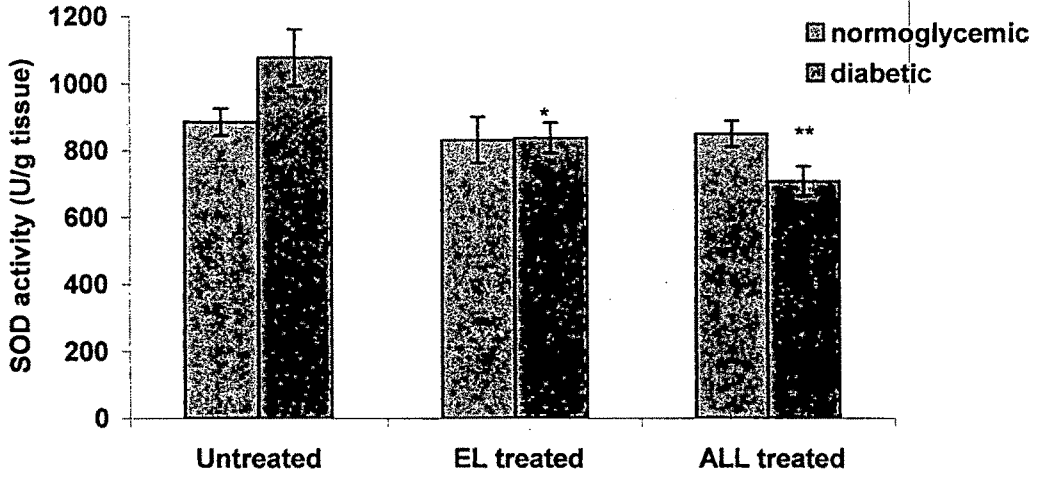
** P< 0.01, *** P< 0.001 as compared to untreated diabetic

Fig 17: Effect of aqueous extract of *E. littorale* and herbal combination (ALL) on liver SOD activity of rats on 20th day



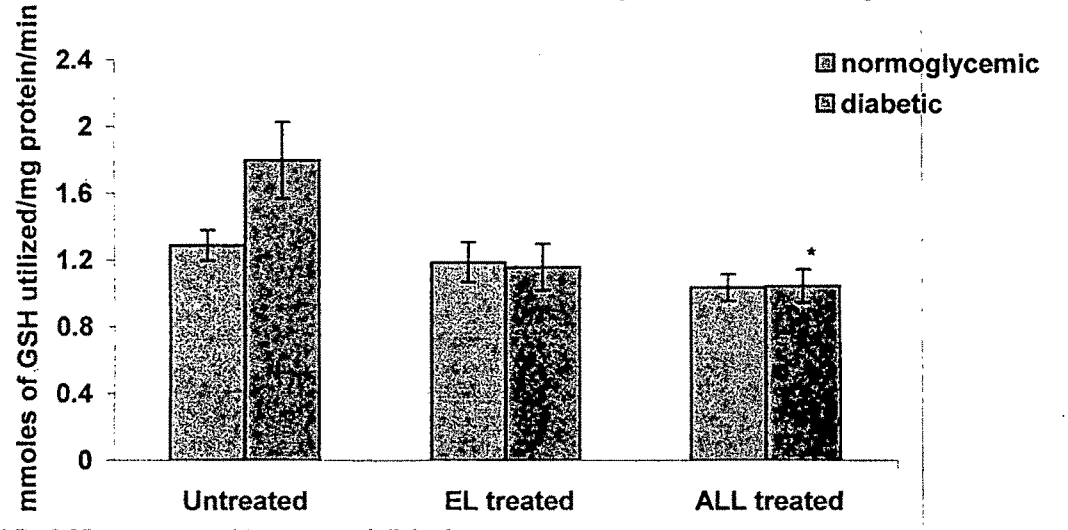
* P< 0.05, ** P< 0.01 as compared to untreated diabetic

Fig 18: Effect of aqueous extract of *E. littorale* and herbal combination (ALL) on kidney SOD activity of rats on 20th day



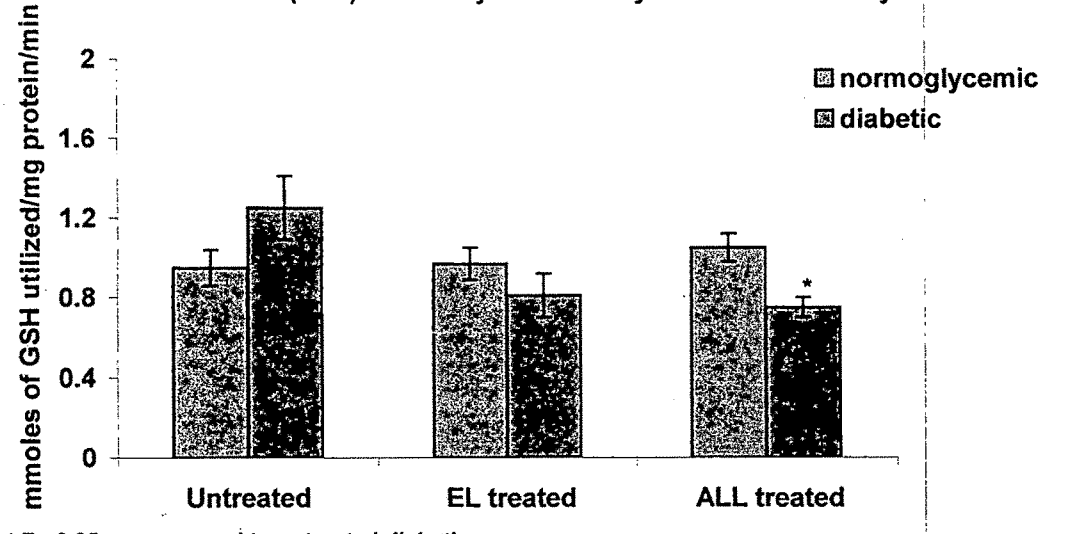
* P< 0.05, ** P< 0.01 as compared to untreated diabetic

Fig 19: Effect of aqueous extract of *E. littorale* and herbal combination (ALL) on liver GPx activity of rats on 20th day



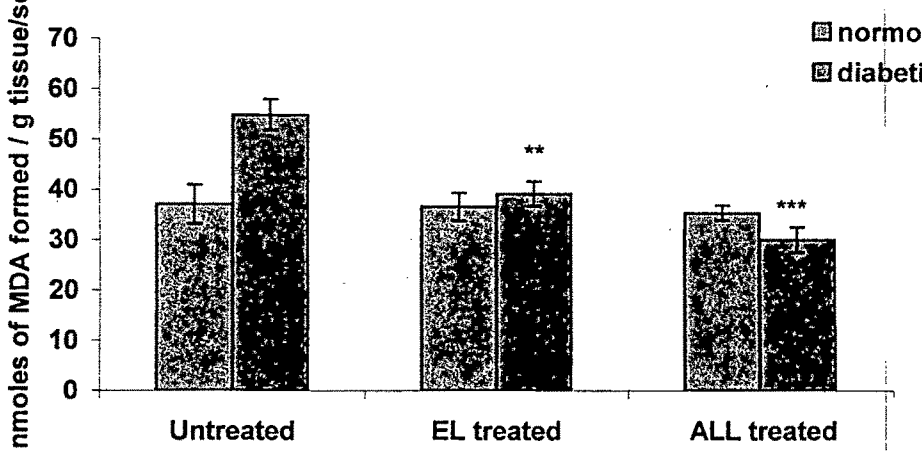
* P< 0.05 as compared to untreated diabetic

Fig 20: Effect of aqueous extract of *E. littorale* and herbal combination (ALL) on kidney GPx activity of rats on 20th day



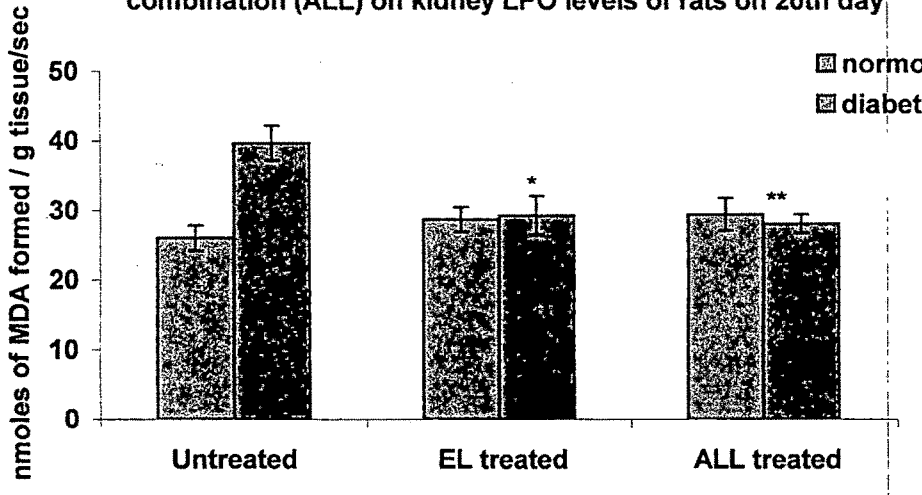
* P< 0.05 as compared to untreated diabetic

Fig 21: Effect of aqueous extract of *E. littorale* and herbal combination (ALL) on liver LPO levels of rats on 20th day



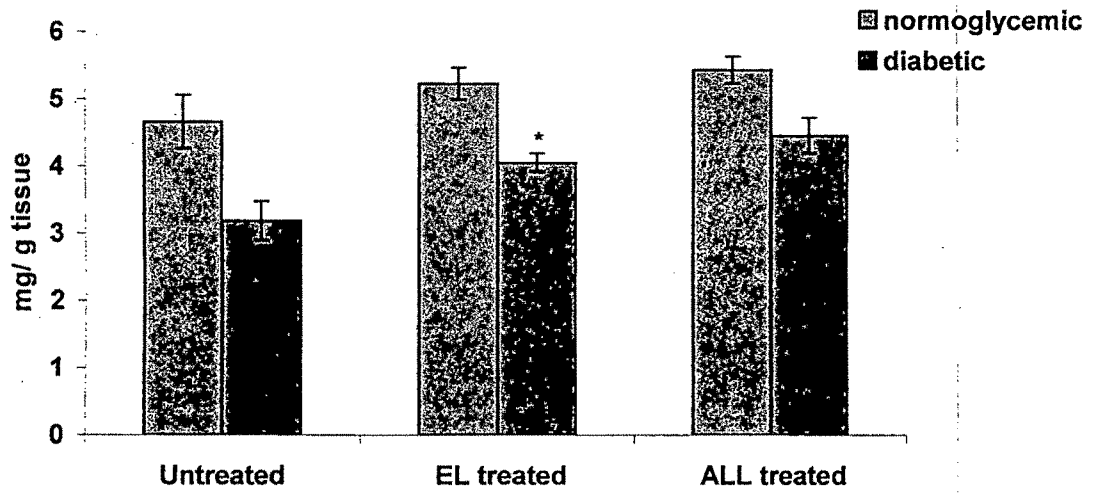
** P< 0.01, *** P< 0.001 as compared to untreated diabetic

Fig 22: Effect of aqueous extract of E. littorale and herbal combination (ALL) on kidney LPO levels of rats on 20th day



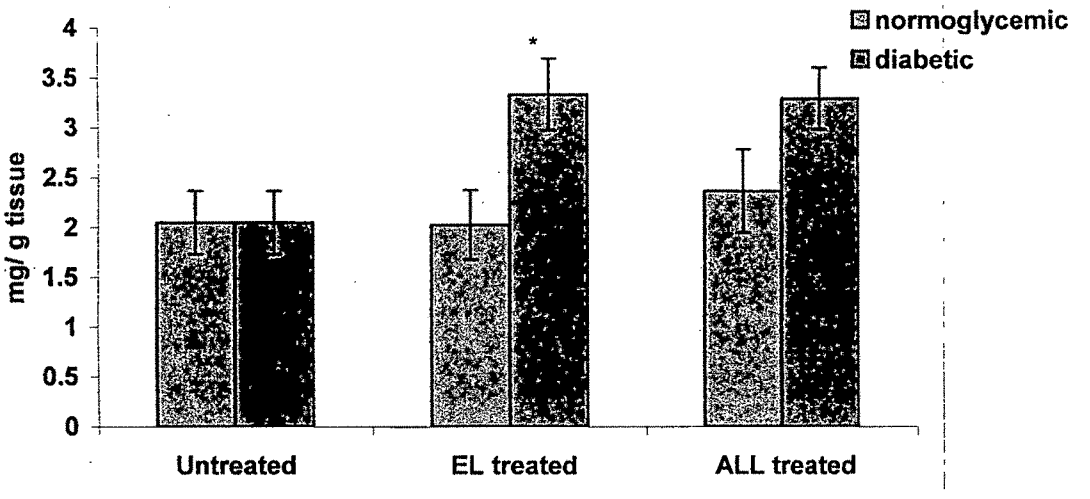
* P< 0.05, ** P< 0.01 as compared to untreated diabetic

Fig 23: Effect of aqueous extract of *E. littorale* and herbal combination (ALL) on liver GSH levels of rats on 20th day



* P< 0.05, ** P< 0.01 as compared to untreated diabetic

Fig 24: Effect of aqueous extract of *E. littorale* and herbal combination (ALL) on kidney GSH levels of rats on 20th day



* P< 0.05 as compared to untreated diabetic

Fig 25: Effect of aqueous extract of *E. littorale* and herbal combination on in vitro DPPH free radical scavenging

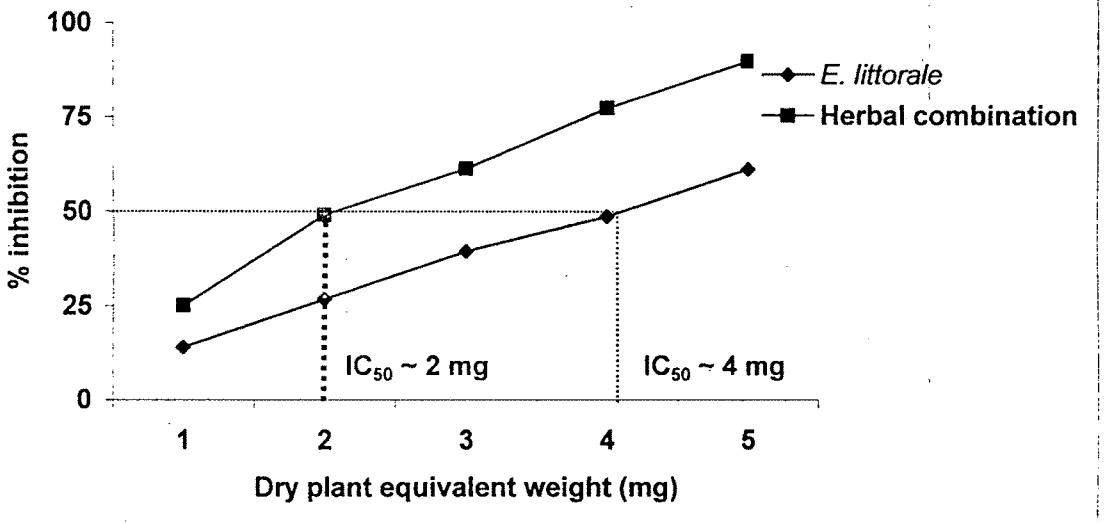


Fig 26: Protective effect of aqueous extract of *E. littorale* (200 µg) and herbal combination (150 µg) on reduced glutathione content in liver homogenate

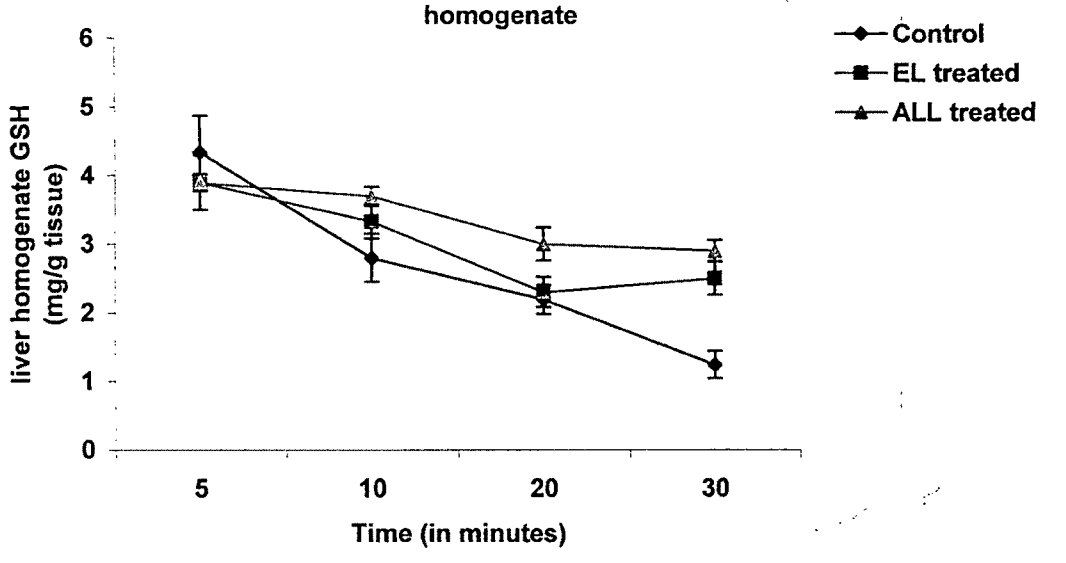
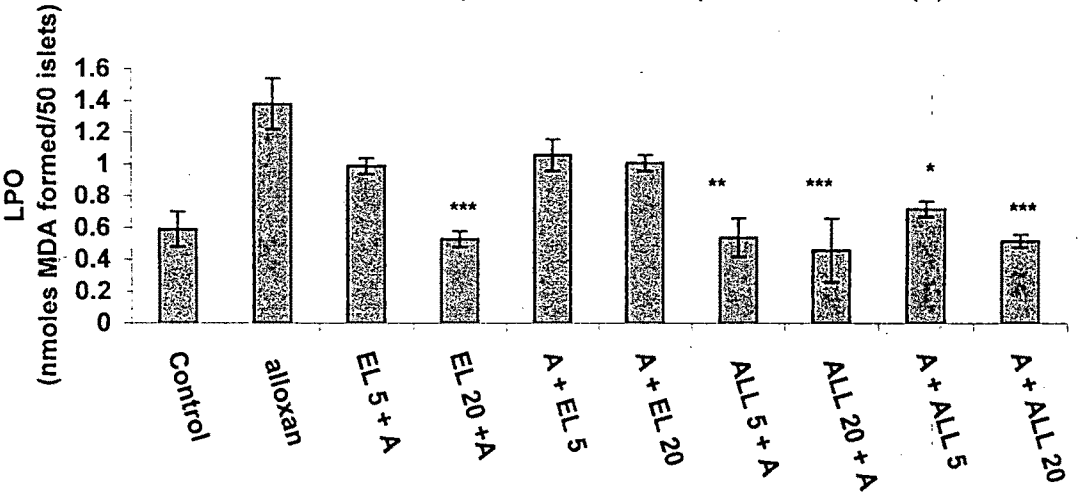
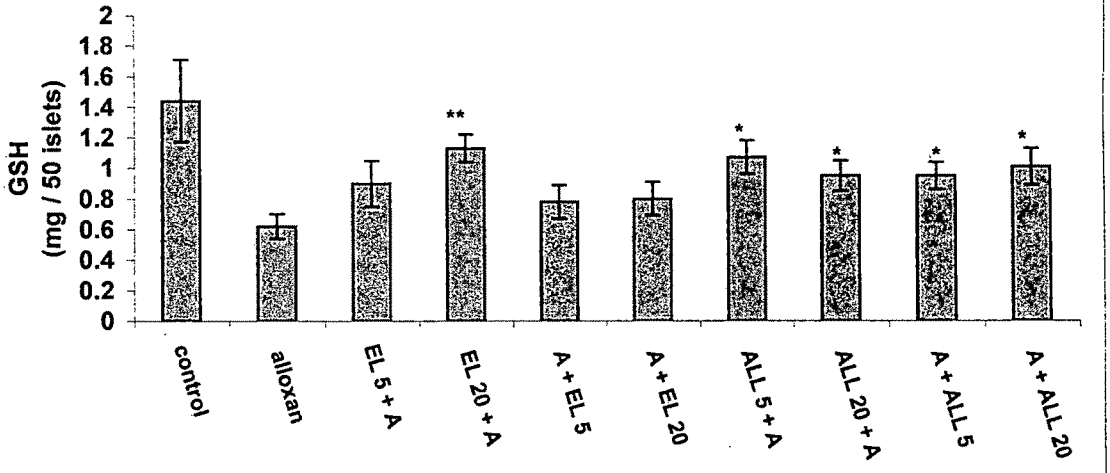


Fig 27: Effect of *E. littorale* (EL) and herbal combination (ALL) on lipid peroxidation in isolated rat pancreatic islets exposed to alloxan (A)



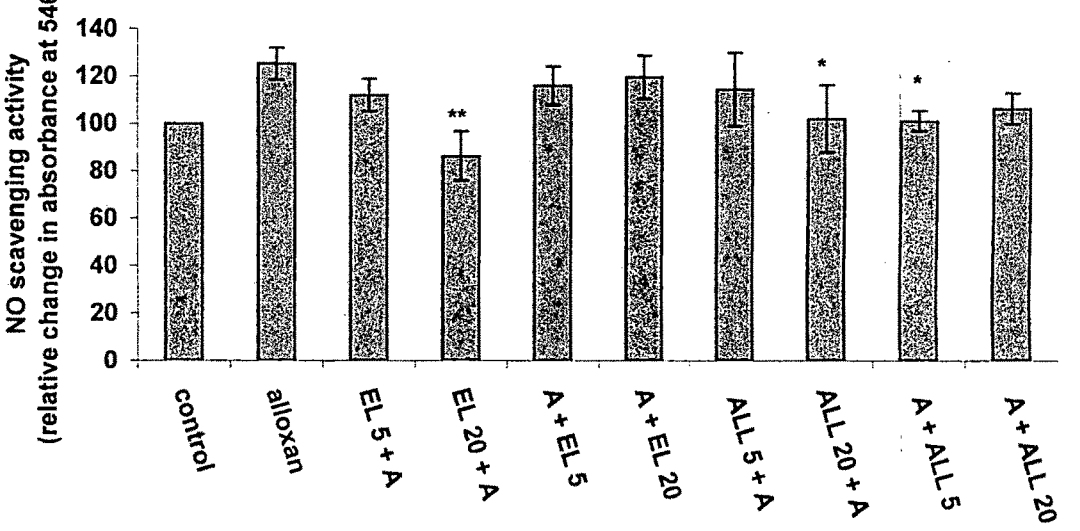
* P< 0.05, ** P< 0.01, *** P< 0.001 as compared to alloxan group

Fig 28: Effect of *E. littorale* (EL) and herbal combination (ALL) on reduced glutathione levels in isolated rat pancreatic islets exposed to alloxan (A)



* P< 0.05, ** P< 0.01 as compared to alloxan group

Fig 29: Effect of *E. littorale* (EL) and herbal combination (ALL) on NO production in isolated rat pancreatic islets exposed to alloxan (A)



* P< 0.05, ** P< 0.01 as compared to alloxan group