Chapter III

Clinical evaluation of antidiabetic efficacy of *E. littorale* aqueous extract in insulin dependent diabetes mellitus (IDDM) and non-insulin dependent diabetes mellitus (NIDDM) patients

- Introduction
- Experimental design
- Results
- Discussion
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Introduction

Diabetes is recognized as one of the highly prevalent disease in India and all over the world with a prevalence rate of 1 – 5 % (Rao et.al., 1989). According to a recent report published by WHO, the countries like India, China and US has the largest number of people with diabetes (King et.al., 1998). Although there are a large number of hypoglycemic drugs available in the market, more and more people are approaching for an alternative treatment for diabetes mellitus in the form of herbal medicine (Bruce and David, 1999). There are reports of using herbal extracts for the treatment of diabetes mellitus in humans (ICMR, 1998). Adverse effects are indeed a cause of concern (Gupta and Raina, 1998), however, available evidence suggests that herbal medicines are relatively safe (Bailey and Day, 1989). Many medicinal plants like *Catha edulis* or "khat" (Saif-Ali et.al., 2003), Psyllium (Sierra et.al., 2002), *Phyllanthus amarus* (Moshi et.al., 2001), *Momordica charantia* (Ahmad et.al., 1999), *Artocarpus heterophyllus* and *Asteracanthus longifolia* (Fernando et.al., 1991), *Gymnema sylvestre* (Baskaran et.al., 1990), *Pterocarpus marsupium* or "Vijayasar" (ICMR, 1998) were reported to be effective in diabetic patients. The plants were not only evaluated for its glucose lowering effect in diabetic patients but also for its antioxidant and hypolipidaemic effect as seen in barley leaf extract (Yu et.al., 2002) and *Morus indica* or "mulberry" (Andallu et.al., 2001) respectively. The beneficial effects of the extract of white-skinned sweet potato (*Ipomoea batatas*), on fasting plasma glucose, as well as on total and low-density lipoprotein (LDL) cholesterol in type 2 diabetic patients were reported which acted by decreasing insulin resistance without affecting body weight, glucose effectiveness, or insulin dynamics. Medicinal plants were also investigated to explore its beneficial effects

against diabetic complications, like *Rhei rhizome* was seen effective on controlling the long-term progression of diabetic nephropathy with overt proteinuria in diabetic patients and prolonged the pre-dialysis period (Goto et.al., 2003), triterpenic fraction of *Centella asiatica* was administered to diabetic patients and found protecting against the deterioration of microcirculation due to diabetic microangiopathy (Cesarone et.al., 2001), *Saptamrita lauha* against diabetic retinopathy (Sharma et.al., 1992). Another study where a combination of *Ginkgo biloba*-extract and folic acid when administered to patients suffering from diabetic neuropathy showed significant improvement in nerve function (Koltringer et.al., 1989).

Enicostemma littorale Blume, commonly known as "mamejua" in Gujarati and "chota chirayita" in Hindi is a medicinal herb of Gentianaceae family and is used by the rural folks of Gujarat (India) as an antidiabetic agent. In an earlier study Vyas et.al. (1979) had shown its hypoglycemic effect in combination with *Mullogo cerviana* in alloxan induced diabetic rabbits. This herb has been systematically been evaluated in our lab and its glucose lowering effect (Vijayvargia et.al, 2000; Maroo et.al., 2003a) and antioxidant effect (Maroo et.al., 2003b) in alloxan-induced diabetic rats with no toxicity was reported. Possible mechanism of glucose lowering effect of aqueous extract of *E. littorale* was seen to be associated with potentiation of glucose-induced insulin release through K^+ – ATP channel dependent pathway but did not require Ca^{2+} influx (Maroo et.al, 2002). Hence it is worthwhile to investigate the antidiabetic efficacy of this herb in diabetic patients. The present study was an attempt to evaluate the hypoglycemic, antioxidant and hypolipidaemic efficacy of the aqueous extract of *E. littorale* in insulin-dependent (IDDM) and non-insulin-dependent (NIDDM) diabetic patients.

Experimental Design

Experiments were carried out in two phases. For both the cases, information on general clinical history and diet of patients volunteered were obtained. Diabetics with fasting blood glucose levels between 150 & 250 mg/dl and postprandial blood glucose levels between 200 & 300 mg/dl were selected for treatment with aqueous extract of *E. littorale*. Presence of any systemic diseases and body mass index (BMI) less than 19 were considered as exclusion criteria. Study protocol was approved by the ethics committee of Baroda Medical College, Vadodara, Gujarat, India. All subjects participating in the study gave their informed written consent.

Phase I Study

In the first phase, a total of 60 diabetes mellitus patients (IDDM : 12; NIDDM : 48) were randomized. IDDM patients were asked to take the aqueous extract of *E. littorale* orally (two divided doses half an hour before meal i.e. 5 g aqueous extract per single dose) along with insulin, designated as EL + Insulin. NIDDM patients taking the aqueous extract were further classified into two – those taking other oral hypoglycemic drugs (OHDs) like sulphonylureas (EL + OHDs) and those who were controlling the disease by diet control, exercise and other conventional therapies but not on any OHDs (EL). The study was carried out for a duration of 5 months. Out of these, 37 successfully completed the trial (EL + I : 8; EL + OHDs : 14; EL : 15) (Table I).

Healthy controls (n=10) also volunteered for the study for a period of 2 months and were self administered the aqueous extract in two divided doses half an hour before meal i.e. 5 g aqueous extract per single dose.

Blood glucose levels were estimated by Glucose-oxidase Peroxidase (GOD-POD) kit method every month and glycosylated haemoglobin levels every two months. Serum lipid profile and antioxidant parameters were estimated at the beginning and end of the study. Serum glutamyl pyruvate transaminase (SGPT), serum alkaline phosphatase (ALP), and serum creatinine levels were estimated for evaluation of toxicity.

Phase II Study

In the second phase, a total of 20 newly diagnosed NIDDM patients were randomized, out of which 11 successfully completed the trial and were taking only *E. littorale* aqueous extract and no other drugs (Table II). The patients were to be orally self administered the aqueous extract in two divided doses, half an hour before meal i.e., 5 g of aqueous extract per single dose. This study was conducted for a period of 2 months. Blood glucose levels were estimated every month and serum samples were immediately stored at –20°C until insulin was determined by Radioimmuno assay (RIA) using a kit from BARC, Mumbai (India). Serum insulin levels, glycosylated haemoglobin levels, serum lipid profile, antioxidant parameters, serum glutamyl pyruvate transaminase (SGPT), serum alkaline phosphatase (ALP) and serum creatinine levels were estimated on 0^{th} and 2^{nd} month.

Results

Phase I Study

Healthy controls

After the end of the treatment, there was no significant change in glycemic parameters (Fig 1 & 2). Though there was a slight improvement in antioxidant parameters (Table III) and serum lipid profile (Fig 3) at the end of the treatment, it was not significant statistically and there was no significant change in the toxicity parameters studied (Table IV).

Diabetic Patients

The aqueous extract treatment showed a decrease in fasting (14%) and postprandial (13%) blood glucose and glycosylated Hb levels (13%) in EL + Insulin group but was not significant statistically. EL + OHDs group showed a significant decrease of 21.1% in fasting and 26.2% in postprandial blood glucose and a decrease of 14.9% in glycosylated Hb levels. EL group showed a significant decrease of 27.8% in fasting, 25.7% in postprandial blood glucose and 17.7% in glycosylated Hb levels at the end of the study (Fig 4 – 6). One way analysis of variance (ANOVA) followed by Tukey's post test did not show any significant difference between the treated groups.

Studies have showed that hyperglycemic state in diabetes mellitus leads to oxidative stress and thus impaired antioxidant status. And dyslipidemia is often associated with diabetes, thus both leading to various complications. At the end of the study, all the groups showed a significant decrease in erythrocyte lipid peroxidation (LPO) levels, erythrocyte catalase (CAT) activity and an increase in blood reduced glutathione (GSH) levels (Fig 7 – 9). At the end of the study, a significant decrease was also seen in all the groups in serum cholesterol levels, serum triglycerides and a significant increase in HDL cholesterol levels after extract treatment (Fig 10 – 12). There was no significant change in serum GPT, ALP and creatinine levels at the end of the study indicating that the extract did not show any toxic effects at this particular dose (Table V).

Phase II Study

With respect to the results obtained from Phase I studies, Phase II study was conducted to see the efficacy of the extract in newly diagnosed NIDDM patients and

were not taking any OHDs or other conventional therapies for a period of 2 months. There was a significant decrease in fasting (31.1%), postprandial (36.3%) and glycosylated Hb (17.7%) levels and an increase in serum insulin levels (32.5%) at the end of the trial as compared to values before treatment (Fig 13 – 15). There was also a significant improvement in all the antioxidant parameters (Table VI) and serum lipid profile (Fig 16) studied as compared to values before treatment. The extract also did not show any toxic effects (Table VII).

Discussion

Earlier our lab had reported the hypoglycemic action of aqueous extract of *E. littorale* in alloxan-induced diabetic rats (Vijayvargia et.al., 2000). It was also shown that the glucose-lowering action of the extract is dependent on plasma glucose concentration with no effect on normoglycaemic rats. Many of the antidiabetic plants had shown similar glucose dependent hypoglycemic action (Ichiki et.al., 1998). The present study was to further investigate the efficacy of the extract in both insulin-dependent (IDDM) and non-insulin dependent (NIDDM) diabetic patients.

Dose of 5 g aqueous extract per single dose, twice a day half an hour before meal, when given to healthy controls did not show any effect in the glycemic parameters, thus supported the fact that the hypoglycemic effect of the aqueous extract of *E. littorale* is dependent on blood glucose concentration. Though there was a decrease of 11.7% in erythrocyte CAT activity, decrease in 16.5% in erythrocyte LPO levels and an increase in 12.8% in blood GSH levels, these were not significant statistically. Similarly, there was also an improvement in serum lipid profile at the end of the two months treatment but

was not significant statistically and was same for the toxicity parameters (SGPT, ALP & serum creatinine) studied.

The aqueous extract treatment in IDDM patients, who were taking insulin and other OHDs therapy did not show any significant change in the glycemic parameters, though a decrease was seen. This can be explained by the fact that patients belonging to IDDM group were not newly diagnosed and were suffering from the disease since long duration (5 – 10 yrs). Earlier *invitro* studies in our lab on isolated rat pancreatic islet cells which were incubated with 11.1 mM glucose along with aqueous extract (20 µg dry plant equivalent) had showed an enhanced glucose induced insulin release (Maroo et al., 2002). Thus the inability of the extract to induce more insulin release due to the already exhausted pancreatic islets may be the reason behind the ineffectiveness in controlling the glycemic index in treated IDDM patients. NIDDM patients who were taking OHDs when treated with the aqueous extract of *E. littorale* showed a decrease of 21.1% in fasting and 26.2% in postprandial blood glucose and a decrease of 14.9% in glycosylated Hb levels. Similarly NIDDM patients who were not on OHDs but other conventional therapies like diet control, exercise and quack remedies when treated with the aqueous extract of *E. littorale* showed a significant decrease of 27.8% in fasting, 25.7% in postprandial blood glucose and 17.7% in glycosylated Hb levels at the end of the 5 month study. This effect may be attributed to increased insulin release from pancreatic islets (Maroo et al., 2002) and/or due to it's effect on increasing insulin sensitivity in streptozotocin-induced NIDDM rats as reported earlier by other workers (Murali et.al., 2002).

For the Phase II study, newly diagnosed NIDDM patients (4 – 8 months) were selected and were administered the *E. littorale* aqueous extract alone for a period of 2

months. There was a significant decrease in all the glycemic parameters studied in NIDDM patients after aqueous extract (*E. littorale*) treatment suggesting it's usefulness in controlling the hyperglycemic state. The decrease in plasma glucose levels in NIDDM patients was observed in both fasting and postprandial levels. Further, when long term effect of the extract was evaluated, it was demonstrated that glycosylated haemoglobin levels were significantly decreased in treated diabetics suggesting a better long term control by the extract. Decrease in glycemic parameters may be attributed to increased glucose uptake and metabolism within cells and by potentiating insulin release from pancreatic beta cells, which is shown in the present results, where there is an increase in serum insulin levels in extract treated diabetic patients. Since, the aqueous extract has more than one active constituents, it is difficult to say which particular component is responsible for the hypoglycemic property.

A significant decrease in serum cholesterol and triglycerides levels were also seen with an increase in HDL cholesterol levels and thus the extract was able to control the hyperlipidaemic state of the NIDDM patients. This could be due to direct effect of some of the chemical constituents present in *E. littorale* on lipid metabolism by affecting absorption, synthesis or utilization of cholesterol. Free radicals and peroxides are clearly involved in the pathogenesis of diabetes mellitus (Wolff, 1993). Catalase (CAT) activity, lipid peroxidation (LPO) and reduced glutathione (GSH) levels in red cells give the measure of the extent of free radical damage inflicted (Pippenger et al., 1998). In the present study, significant decrease in erythrocyte CAT activity and LPO levels and an increase in GSH levels were also observed after the administration of extract in the diabetic patients, which could be attributed to the effect of the aqueous extract of the herb

as a potent free radical scavenger. Since hypolipidaemic and antioxidant property has been observed in control as well as in IDDM patients, these effects are not secondary by primary in nature.

Comparing the Phase I and Phase II study it can be seen that the later, where newly diagnosed NIDDM patients had volunteered was more effective than the first. This shows the effectiveness of the plant extract in controlling the disease in an early stage. Though there was no significant decrease in glycemic parameters of IDDM patients, the extract administration demonstrated significant decrease in antioxidant and lipidemic parameters. The present study shows that even when administered alone the extract was very effective in newly diagnosed NIDDM patients and was able to decrease the hyperglycemic and hyperlipidaemic condition significantly and also improved the antioxidant parameters without any toxic effect at this particular dose. Hence, it is a potential candidate for the development of a novel therapeutic agent for NIDDM patients. Though there was no toxicity at the particular dose in both the study, further studies ought to be carried out to see the interaction of the plant extract while given in combination with other OHDs, since drug-herb interactions possibly might exist (Miller, 1998) and should be screened for.

Summary

E. littorale aqueous extract treatment (orally self administered the aqueous extract in two divided doses, half an hour before meal i.e., 5 g of aqueous extract per single dose) to IDDM patients along with insulin showed decrease in glycemic parameters but was not significant statistically. But the extract treatment in NIDDM patients who were on OHDs and other conventional therapies respectively showed significant decrease in fasting and

postprandial blood glucose levels and glycosylated haemoglobin levels. A 2 month extract treatment in newly diagnosed NIDDM patients, who were administered the extract alone showed significant decrease in the glycemic parameters studied with and increase in serum insulin levels. The extract treatment didn't show any decrease in glycemic parameters in healthy controls with no toxic effect in any of the groups studied at this particular dose.

A significant decrease in elevated levels of serum cholesterol levels, serum triglycerides was observed after extract treatment in all the groups with an increase in HDL cholesterol levels at the end of the study. But the effect was seen in healthy controls as well, it was not significant statistically.

Aqueous extract of *E. littorale* also showed significant antioxidant property in all the groups of diabetic patients by increasing blood GSH levels, decreasing erythrocyte LPO levels and CAT activity.

Table I : Details of IDDM and NIDDM patients volunteered for the study

	IDDM (Insulin + Extract)	**NIDDM** (OHDs + Extract)	**NIDDM** (Other therapies + Extract)
Duration of disease	5 – 10 years	5 – 10 years	5 – 10 years
Age (range)	34 - 72 yrs	34 - 72 yrs	34 - 72 yrs
Body weight (range)	48 - 80 kgs	48 - 80 kgs	48 - 80 kgs
Duration of study	5 Months	5 Months	5 Months
Dose of extract	**5 g /single dose twice a day half an hour before meal.**		
Patients Volunteered	12	26	22
Dropouts	04	05	07
Not eligible due to (a) Uncontrolled FBS / PP2BS	0	03	01
(b) Irregularity in taking extract	0	04	09
Patients in analysis	08	14	15

Consent form was procured from each patient

Table II : Details of newly diagnosed NIDDM patients volunteered for the study

	NIDDM (Extract)
Duration of disease	4 – 8 months
Age (range)	38 - 55 yrs
Body weight (range)	48 - 80 kgs
Duration of study	2 Months
Dose of extract	**5 g /single dose twice a day half an hour before meal.**
Patients Volunteered	20
Dropouts	04
Not eligible due to	
(a) Uncontrolled FBS / PP2BS	01
(b) Irregularity in taking extract	04
Patients in analysis	11

Consent form was procured from each patient

Table III. Antioxidant effect of *E. littorale* Blume aqueous extract in healthy volunteers

	Before Treatment	After Treatment
Erythrocyte CAT activity (k/g Hb)	163.4 ± 5.42	144.3 ± 7.54 ^{NS}
Erythrocyte LPO levels (nmoles of MDA formed/g Hb)	112.5 ± 7.30	93.9 ± 5.40 ^{NS}
Blood GSH levels (mg /dl)	45.89 ± 3.16	51.76 ± 2.06 ^{NS}

Values presented as Mean ± SE (n = 10)
NS (non-significant)

Table IV. Effect of *E. littorale* Blume aqueous extract on serum GPT, ALP and creatinine levels in healthy volunteers

	Before Treatment	After Treatment
SGPT (IU/L)	28.74 ± 8.43	29.62 ± 6.40 ^{ns}
ALP (IU/L)	44.56 ± 3.33	42.10 ± 4.01 ^{ns}
Creatinine (mg/dl)	0.43 ± 0.12	0.52 ± 1.15 ^{ns}

(IU = μ M product formed/min)

Values presented as Mean ± SE (n = 10)

Ns = non-significant as compared to values before treatment

Table V. Effect of *E. littorale* Blume aqueous extract on serum GPT, ALP and creatinine levels in diabetic patients

		Before Treatment	After Treatment
IDDM (EL + Insulin) (n = 8)	SGPT (IU/L)	44.15 ± 6.78	46.78 ± 9.99^{NS}
	ALP (IU/L)	89.90 ± 5.32	92.11 ± 9.43 ^{NS}
	Creatinine (mg/dl)	1.12 ± 0.45	1.22 ± 0.38 ^{NS}
NIDDM (EL + OHDs) (n = 14)	SGPT (IU/L)	50.25 ± 7.48	49.18 ± 6.69 ^{NS}
	ALP (IU/L)	73.10 ± 6.98	70.16 ± 8.79 ^{NS}
	Creatinine (mg/dl)	0.98 ± 0.34	1.01 ± 0.18 ^{NS}
NIDDM (EL) (n = 15)	SGPT (IU/L)	43.19 ± 5.55	41.09 ± 3.39 ^{NS}
	ALP (IU/L)	66.78 ± 8.32	67.92 ± 5.90 ^{NS}
	Creatinine (mg/dl)	0.76 ± 0.11	0.79 ± 0.13 ^{NS}

(IU = μ M product formed/min)

Ns = non-significant as compared to values before treatment

Table VI. Antioxidant effect of *E. littorale* Blume aqueous extract in NIDDM patients

	Before Treatment	After Treatment
Erythrocyte CAT activity (k/g Hb)	290.4 ± 10.78	165.6 ± 7.30 ***
Erythrocyte LPO levels (nmoles of MDA formed/g Hb)	210.9 ± 6.02	179.7 ± 11.31 *
Blood GSH levels (mg /dl)	21.75 ± 1.10	28.19 ± 1.76 **

Values presented as Mean ± SE (n = 11)

* P < 0.05, ** P < 0.01, *** P < 0.001 as compared to value before treatment.

Table VII. Effect of *E. littorale* Blume aqueous extract on serum GPT, ALP and creatinine levels in NIDDM patients

	Before Treatment	After Treatment
SGPT (IU/L)	39.4 ± 8.43	38.62 ± 7.44 ^{ns}
ALP (IU/L)	84.6 ± 4.90	90.70 ± 8.03 ^{ns}
Creatinine (mg/dl)	0.74 ± 0.13	0.88 ± 0.29 ^{ns}

(IU = μ M product formed/min)

Values presented as Mean ± SE (n = 11)

Ns = non-significant as compared to values before treatment

Fig 1: Effect of *E. littorale* Blume aqueous extract on blood glucose levels in healthy volunteers

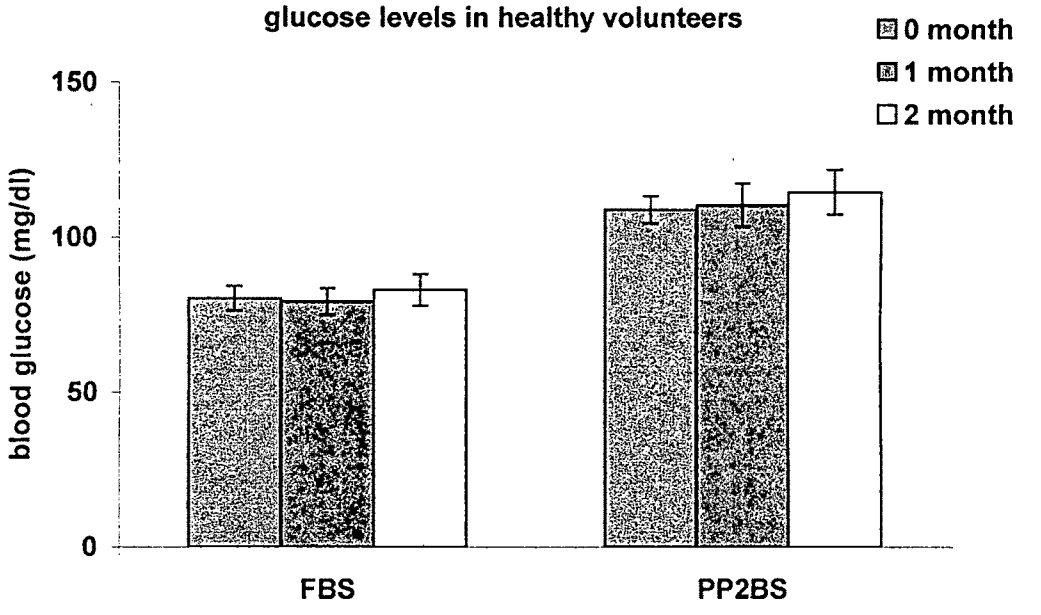


Fig 2: Effect of *E. littorale* Blume aqueous extract on glycosylated haemoglobin levels in healthy volunteers

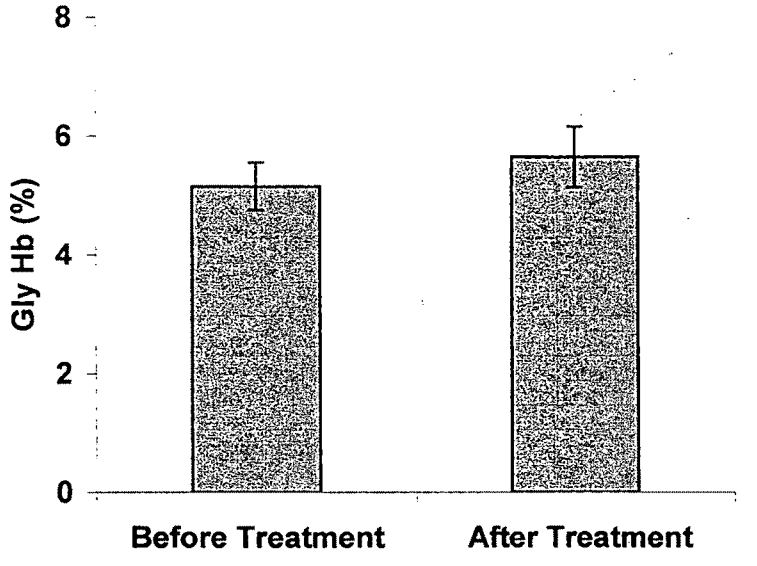


Fig 3: Effect of *E. littorale* Blume aqueous extract on serum lipid profile in healthy volunteers

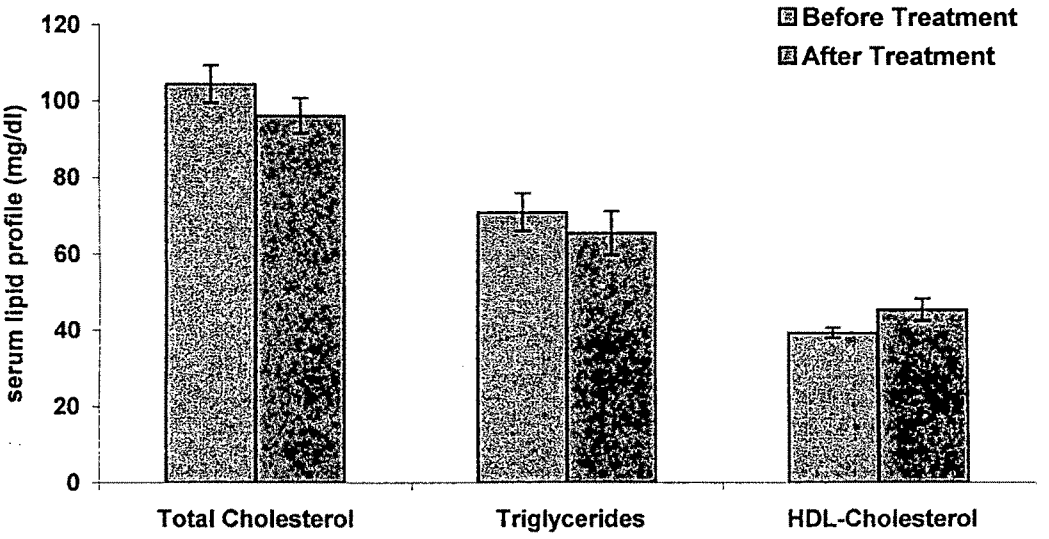


Fig 4: Effect of *E. littorale* Blume aqueous extract on FBS in diabetic patients

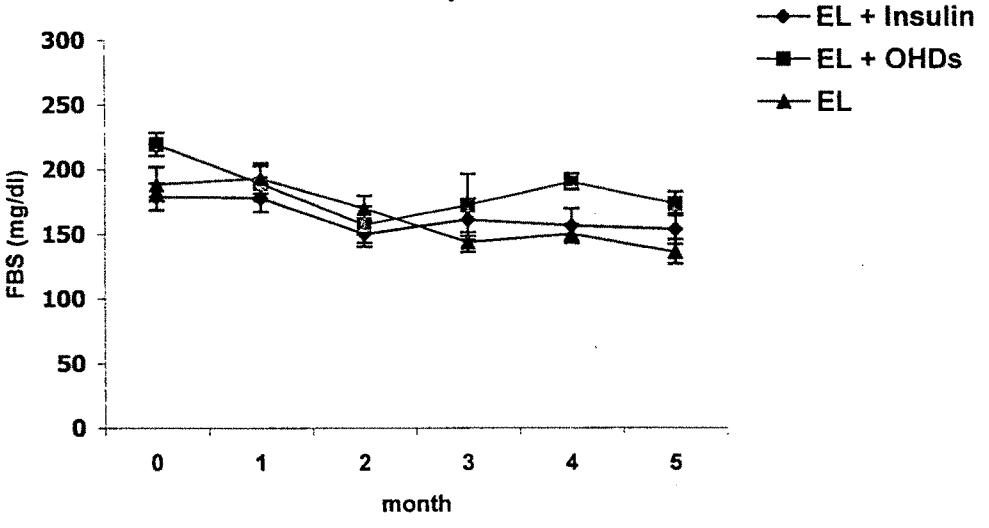


Fig 5: Effect of *E. littorale* Blume aqueous extract on PP_2BS in diabetic patients

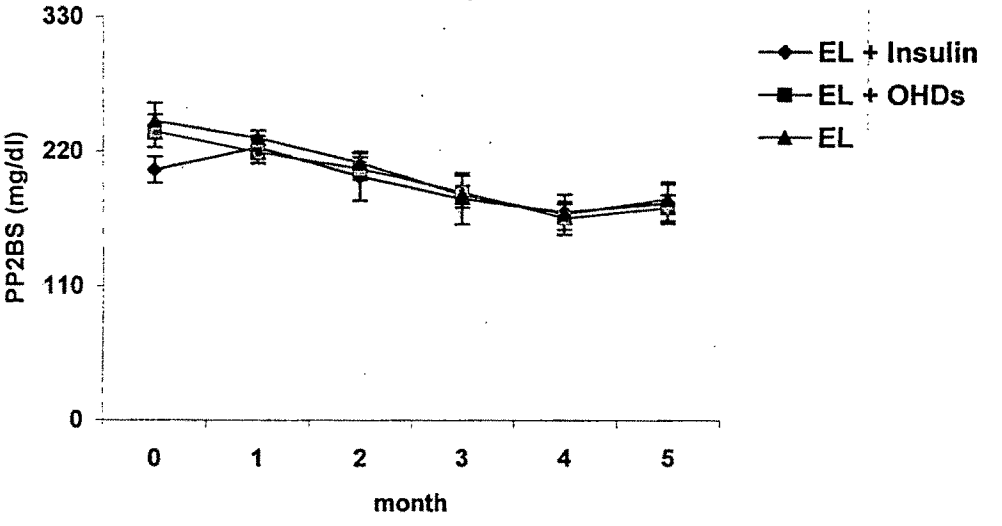
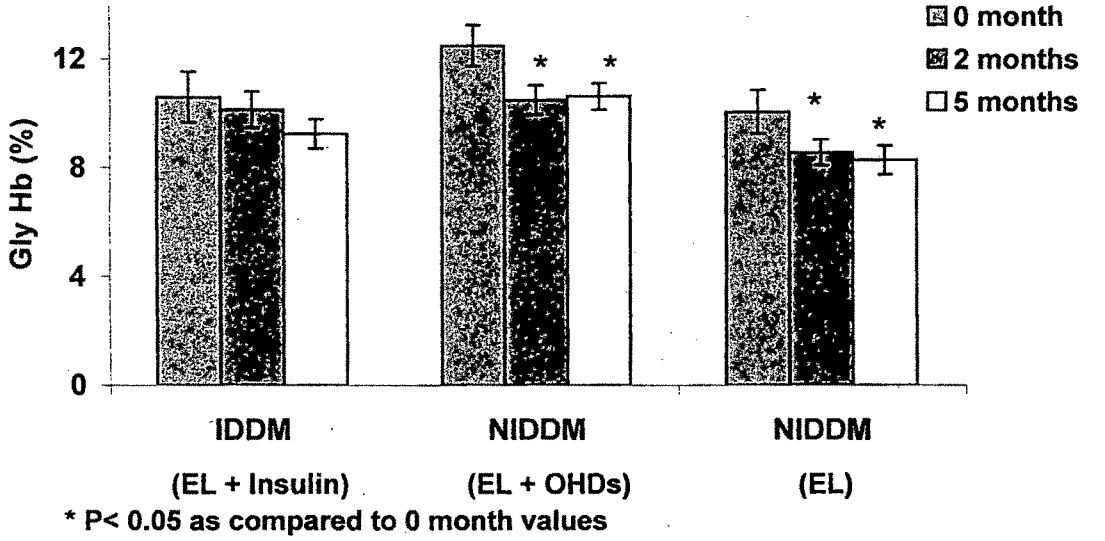


Fig 6: Effect of *E. littorale* Blume aqueous extract on glycosylated haemoglobin levels in diabetic patients



* P< 0.05 as compared to 0 month values

Fig 7: Effect of *E. littorale* Blume aqueous extract on erythrocyte CAT activity in diabetic patients

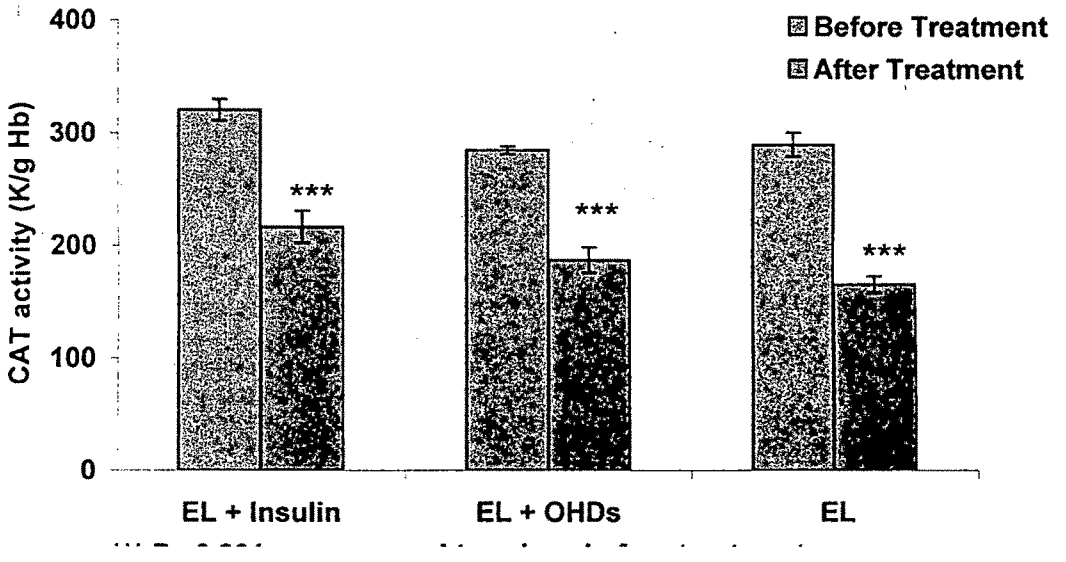


Fig 8: Effect of *E. littorale* Blume aqueous extract on erythrocyte LPO levels in diabetic patients

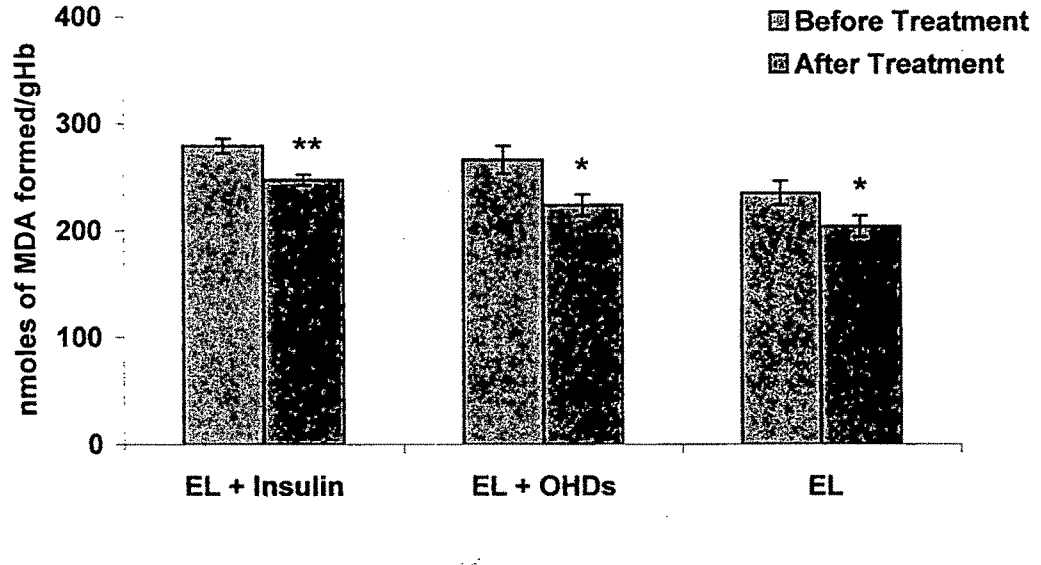


Fig 9: Effect of *E. littorale* Blume aqueous extract on blood GSH levels in diabetic patients

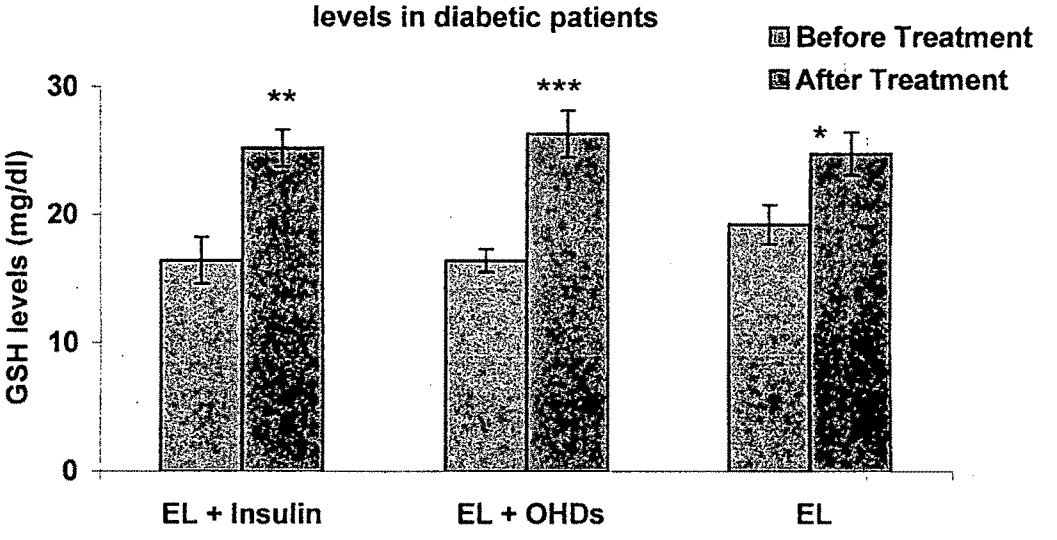
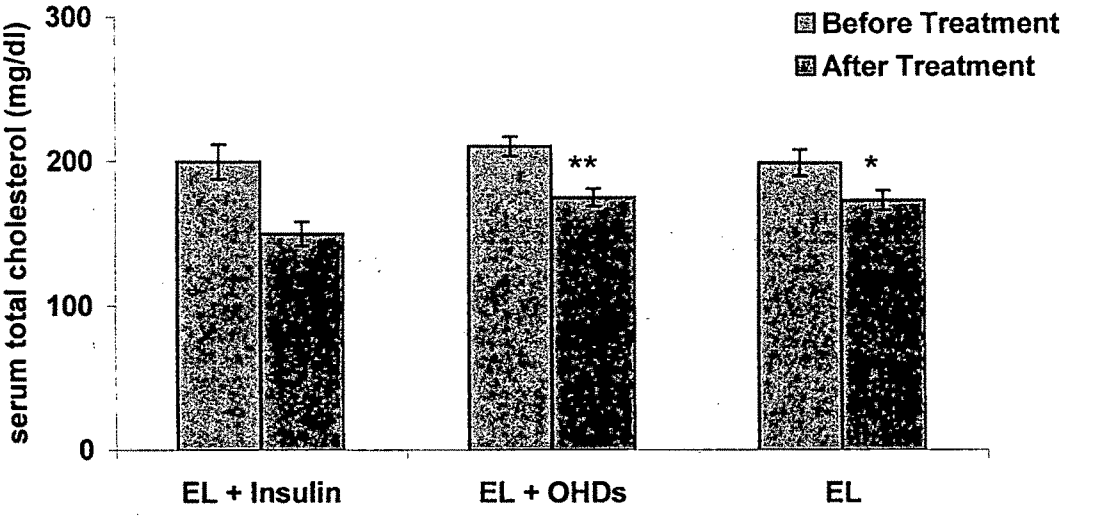
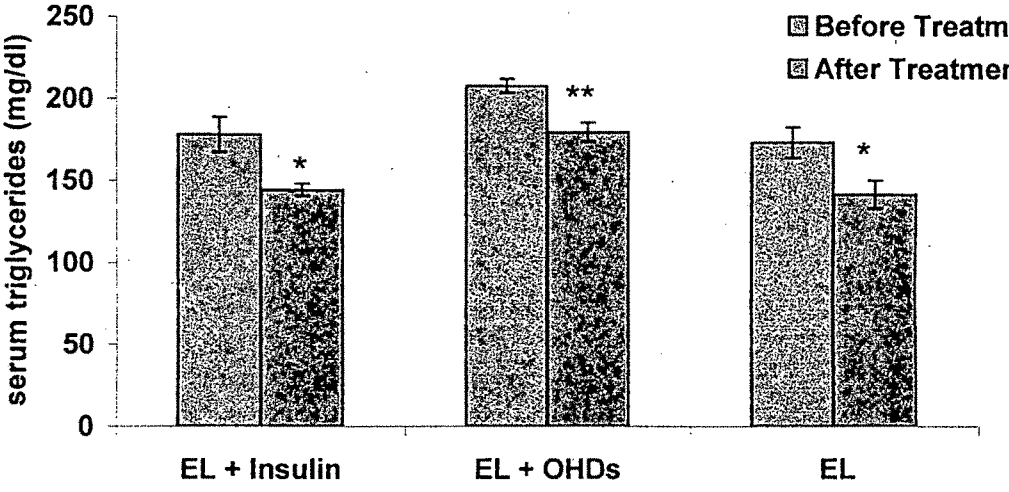


Fig 10: Effect of *E. littorale* Blume aqueous extract on serum total cholesterol levels in diabetic patients



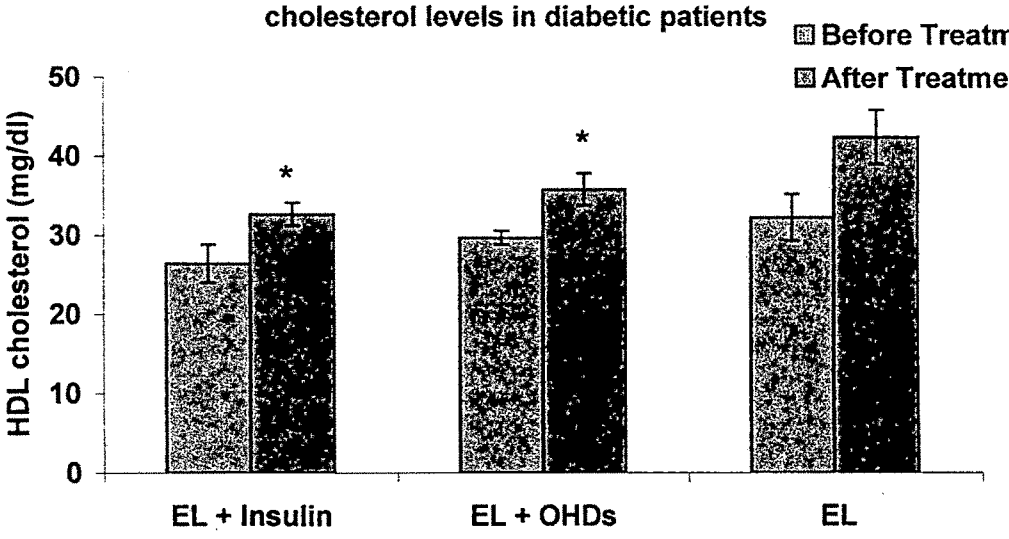
* P<0.05, ** P<0.01 as compared to values before treatment

Fig 11: Effect of *E. littorale* Blume aqueous extract on serum triglycerides levels in diabetic patients



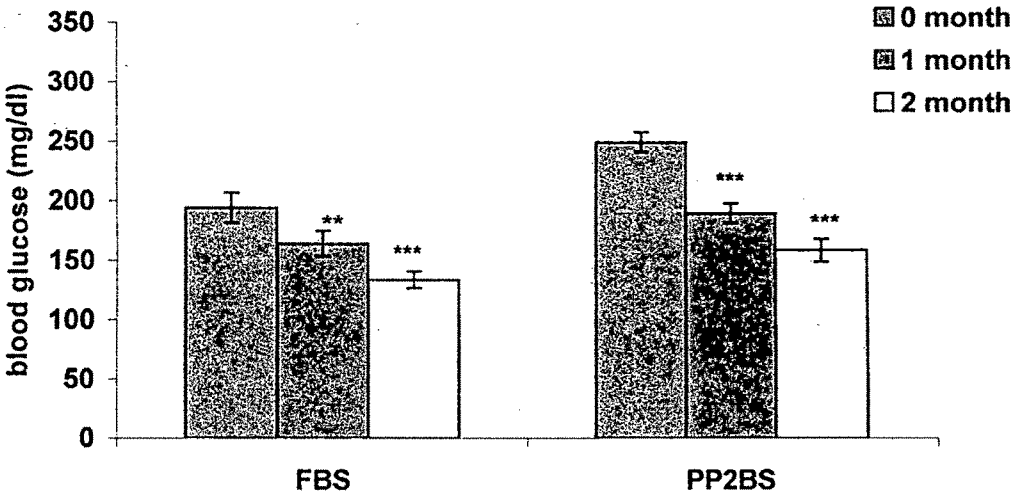
* P<0.05, ** P< 0.01 as compare to values before treatment

Fig 12: Effect of *E. littorale* Blume aqueous extract on HDL cholesterol levels in diabetic patients



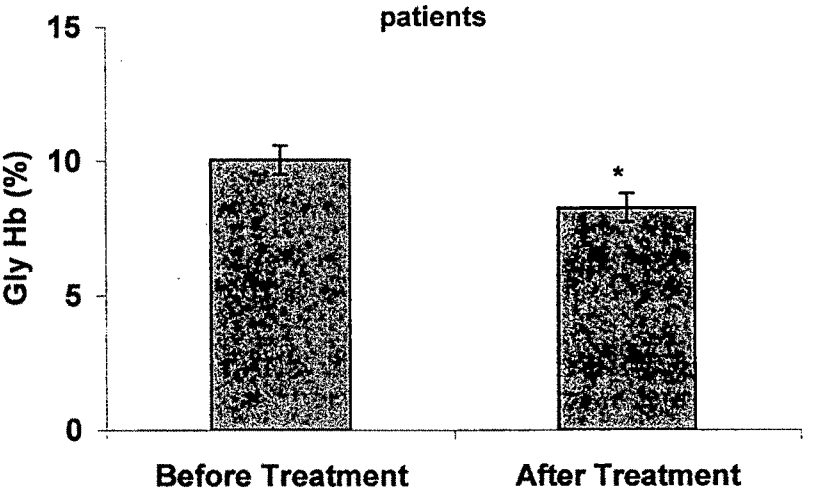
* P< 0.05 as compared to values before treatment

Fig 13: Effect of *E. littorale* Blume aqueous extract on blood glucose levels in newly diagnosed NIDDM patients



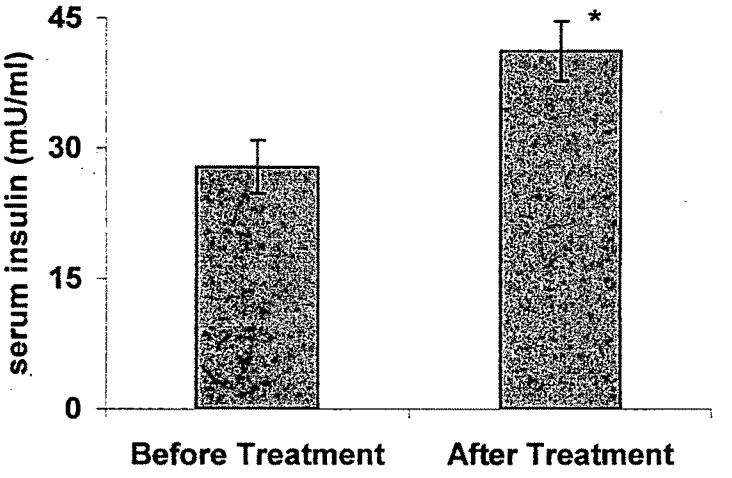
** P< 0.01, *** P < 0.001 as compared to values before treatment

Fig 14: Effect of *E. littorale* Blume aqueous extract on glycosylated hemoglobin levels in newly diagnosed NIDDM patients



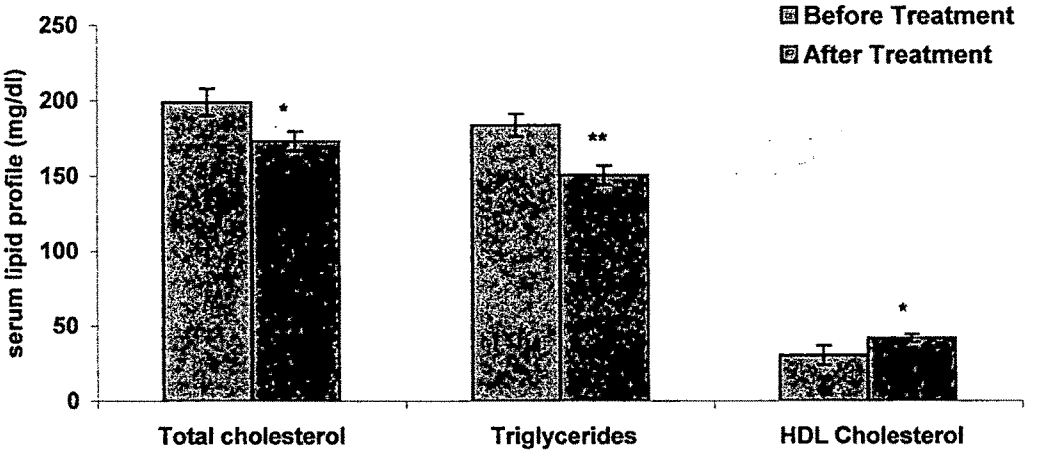
* P< 0.05 as compared to values before treatment

Fig 15: Effect of *E. littorale* Blume aqueous extract on serum insulin levels in newly diagnosed NIDDM patients



* P< 0.05 as compared to values before treatment

Fig 16: Effect of *E. littorale* Blume aqueous extract on serum lipid profile in newly diagnosed NIDDM patients



* P< 0.05, ** P< 0.01 as compared to values before treatment