Chapter IV

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

- Introduction
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Introduction

Despite the great strides that have been made in understanding and management of diabetes mellitus, serious complications continue to confront patients and physicians. The graph of diabetes related mortality is rising unabated (Olefsky, 2000). Prolonged exposure to pancreatic islets to elevated glucose concentration has been shown both *in vitro* and *in vivo* to impair glucose-stimulated insulin release (Leaky and Weir, 1988). The unidirectional therapeutic approach in the management of diabetes does not appear to be the way to address this problem. Most of the herbal preparations or "Ayurvedic medicines" available in the market for various ailments are combinations of various medicinal plants. Though care and scientific credence ought to be obtained before administration to the ailing patients, most of them take it own there own without consulting the doctors. Studies are going on to evaluate the various herb's efficacy when given in combination so as to increase its efficacy and at the same time to investigate for possible herb-herb interaction leading to toxicity if any. The anti-hyperglycemic effect of a herbal preparation "antidiabetis" consisting of 10 medicinal plants showed significant decrease in serum glucose and fructosamine levels in alloxan-induced NOD mice (Petlevski et.al., 2001). Similarly, D-400, a herbomineral preparation showed considerable lowering of AUC in oral glucose tolerance test, triglycerides, HDL cholesterol and a rise in hepatic glycogen levels in streptozotocin-induced diabetic rats. In the pancreas of diabetic rats, D-400 therapy showed significant increase in islet number and beta cell count and appeared to bring about blood sugar homoeostasis by increasing insulin secretion and repair/regeneration of endocrine pancreas (Mitra et.al., 1996). Effect of Cogent db, a compound herbal drug was reported to significantly reduce blood glucose, glycosylated haemoglobin and increased plasma insulin, total haemoglobin along with antihyperlipidemic effects in alloxan-induced diabetic rats (Pari and Saravanan, 2002) and the same effects were seen in diabetic patients without any side effects (Shekhar et.al., 2002). "Diabetin", a phytopreparation showed sugar-reducing, anorexigenic effects and caused the manifestations of diabetic angio- and neuropathies to delay (Bodnar et.al., 2000). *Medicatrix naturae* – the power of self preservation or adjustment has been the motto of traditional medicinal practice, which prescribes polyherbal formulations (Tiwari and Rao, 2002). The theories of polyherbal formulation have the synergistic, potentiative, agonistic/antagonistic pharmacological agents within themselves due to incorporation of plant medicines with diverse pharmacological actions. These pharmacological principles work together in a dynamic way to produce maximum therapeutic efficacy with minimum side effects.

For the present study, a combination of *Curcuma longa* dry rhizome, *Emblica officinalis* dry fruit, *Trigonella foenum-graecum* dry seeds was used along with dry whole plant of *Enicostemma littorale*. The selected medicinal plants were commonly found in the region and were used in almost every antidiabetic preparations. The effect of *C. longa* or turmeric and its active principle curcumin was found to significantly decrease blood sugar, Hb and glycosylated hemoglobin levels (Arun & Nalini, 2002). The hypoglycemic and hypolipidaemic properties of the flavonoids of *Emblica officinalis* or amala was reported (Anila & Vijayalakshmi, 2000). Blood glucose was reduced by an aqueous extract of Trigonella leaf in rats whereas ethanolic extract was only active by ip administration (Abdel-Barry et.al., 1997). The seeds of *Trigonella foenum-graecum* or "methi" have 4-hydroxyisoleucine, which increases glucose-induced insulin release, at

100-1000 microM and is ineffective at low (3 mmol/l) or basal (5 mmol/l) glucose concentrations (Sauvaire et.al., 1998). Our lab had reported the glucose lowering effect of *Enicostemma littorale* (Vijayvargia et.al, 2000; Maroo et.al., 2003a) in alloxan-induced diabetic rats and had reported it's possible mechanism to be associated with potentiation of glucose-induced insulin release (Maroo et.al, 2002). Its effect on increasing insulin sensitivity in streptozotocin-induced (NIDDM) diabetic rats was also reported by other workers (Murali et.al., 2002).

Thus the present study was carried out to see the combined effect of *Curcuma longa*, *Emblica officinalis*, *Trigonella foenum-graecum* and *Enicostemma littorale* in alloxan-induced diabetic rats and compare with that of *E. littorale* aqueous extract.

Experimental Design

Qualitative chemical analyses

The qualitative chemical analysis of the aqueous extract of *E. littorale* and herbal combination were done according to standard methods.

Experiments were carried out both *in-vivo* and ex-*vivo*. *In-vivo* experiments were further divided into – short-term experiment and long-term experiment. Toxicity parameters were assessed in long-term experiments.

***In-vivo* experiment**

(i) Short-term experiment

After 12-14 hours of food deprivation, fasting blood samples were collected from normoglycemic and alloxan-induced diabetic rats. Treated groups of rats received a single dose of aqueous extract (1.5 g dry plant equivalent extract/100g body weight) of herbal combination and *E. littorale* aqueous extract, and the untreated control rats received equal amount of tap water via gastric intubation. Glibenclamide (0.25mg/100g b. wt.) was used as reference drug. After the treatment blood samples were collected from orbital sinus at intervals of 2, 4 and 8 hours. Plasma glucose was estimated by Glucose-Oxidase Peroxidase (GOD-POD) kit method and serum samples were immediately stored at –20°C until insulin was determined by Radio-Immuno Assay (RIA) kit (BARC, Mumbai, India).

(ii) Long-term experiment

Animals were made diabetic by injecting alloxan as described earlier. Further, animals were divided into four major groups – untreated normoglycemic, treated normoglycemic, untreated diabetic and treated diabetic. Animals received aqueous extract (1.5g dry plant equivalent extarct/100g body weight/day) of herbal combination and *E. littorale* aqueous extract for 20 days respectively. Blood samples were collected at 0^{th}, 10^{th} and 20^{th} day. Plasma glucose levels were estimated on 0, 10^{th} and 20^{th} day, glycosylated Hb and serum insulin levels on 0^{th} and 20^{th} day. Serum glutamyl pyruvate transaminase (SGPT), serum alkaline phosphatase (ALP), serum creatinine levels and liver GPT and ALP levels were also estimated by sacrificing the animals on 20^{th} day for evaluation of toxicity.

***Ex-vivo* experiment**

Ex-vivo experiments were performed with isolated rat pancreatic islets to monitor insulin release potential of aqueous extract of herbal combination by incubating the extract with isolated rat pancreatic islets as described in chapter II. 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 11.1mM glucose and concentrations of 10 and 20 μg dry plant equivalent of *E. littorale* (Maroo et.al., 2002) and herbal combination extracts.

Results

The qualitative analysis of the aqueous extract of *E. littorale* and herbal combination showed the presence of various compounds as presented in Table I.

Short-term experiment

In the short term experiments, administration of aqueous extracts of *E. littorale* (EL) and herbal combination (ALL) did not show any effect on blood glucose and serum insulin levels in normoglycaemic rats. But, glibenclamide treated groups showed significant decrease in blood glucose levels (P< 0.001) and increase in serum insulin levels (P< 0.05) at 2nd, 4th and 8th hour of administration as compared to 0th hr in normoglycaemic rats (Fig 1, 2). In alloxan-induced diabetic rats, herbal combination showed much earlier response in lowering blood glucose at 2nd hr, against *E. littorale* extract which showed a late response at 4th hr of administration (Fig 3). The results were similar with regard to serum insulin levels (Fig 4).

Long-term experiment

E. littorale and herbal combination extract treatment to normoglycaemic rats for a period of 20 days did not show any significant change in blood glucose, serum insulin or glycosylated haemoglobin levels (Fig 5 – 7). But there was a significant decrease in blood glucose, glycosylated haemoglobin levels and increase in serum insulin levels in *E. littorale* and herbal combination extract treated diabetic rats at 10th and 20th day, as compared to 0th day values (Fig 8 – 10). Comparing *E. littorale* and herbal combination, the latter was found to be more effective in controlling hyperglycemia.

The toxicity parameters like serum GPT, ALP, serum creatinine levels and liver GPT and ALP levels did not show any significant change in extract treated normoglycaemic and diabetic rats (Table II).

***Ex-vivo* experiment**

Ex-vivo experiments with aqueous extracts of *E. littorale* and herbal combination on insulin release from isolated rat pancreatic islets in presence of 11.1 mM glucose showed a significant increase in insulin release in both 10 and 20 μg concentrations as compared to untreated control (Fig 11).

Discussion

Diabetes mellitus is characterized by the classical symptom of "hyperglycemia" which in turn leads to various complications if left untreated. A lot of antidiabetic plants have been reviewed and *C. longa*, *E. officinalis*, *T. foenum-graecum* and *E. littorale* selected for the present study had shown potent glucose lowering effect in diabetic conditions. A single dose of 1.5 g dry plant equivalent extract/100g body weight was used for the study, as this was found to be the effective dose of *E. littorale* as reported earlier (Maroo et.al., 2003b). Aqueous extract of *E. littorale* when administered to normoglycaemic rats for 8 hrs did not show any hypoglycemic effect as reported earlier (Vijayvargia et.al., 2000). Herbal combination also demonstrated similar effect. Whereas there was a significant decrease in blood glucose levels in glibenclamide treated normoglycaemic rats. When the extracts were administered to alloxan-induced diabetic rats, herbal combination showed a significant decrease in blood glucose levels from the 2^{nd} hr of administration as against *E. littorale*, which showed a delayed response from 4^{th} hr, as compared to 0 hr blood glucose values. The above results shows that by combining the selected medicinal plants, the delayed response which was shown by *E. littorale* alone, was decreased and thus the potency of the hypoglycemic effect increased. At the same time, the combination treatment did not show any effect on normoglycaemic rats and thus increasing its therapeutic value.

In the case of long term experiments, there was no significant change in blood glucose, serum insulin or glycosylated haemoglobin levels, in both *E. littorale* and herbal combination treated normoglycaemic rats, which were supportive of the short term experiments. In diabetic rats, both the extract treatment for 20 days caused significant decrease in blood glucose and glycosylated haemoglobin levels and a significant increase in serum insulin levels at the end of the treatment as compared to 0^{th} day. Comparatively the effect was seen more in the herbal combination. Administration of *C. longa* or its active principle, curcumin to alloxan-induced diabetic rats was reported to significantly reduce blood sugar and glycosylated hemoglobin levels and had also showed inhibitory activity of sorbitol dehydrogenase enzyme (Arun and Nalini et.al., 2002). *Trigonella foenum graecum* administration to diabetic rabbits produced significant attenuation of the glucose tolerance curve and improvement in the glucose induced insulin response, suggesting that the hypoglycemic effect may be mediated through stimulating insulin synthesis and/or secretion from the beta pancreatic cells of Langerhans (Puri et.al., 2002), moreover, 4-hydroxy isoleucine, an insulin secretagougue was also isolated from the seeds of fenugreek (Sauvaire et.al., 1998). Fenugreek is also reported to modulate the carbohydrate metabolizing enzymes like glucokinase (GK), hexokinase (HK), and phosphofructokinase (PFK) in diabetic condition (Vats et.al., 2003). The hypoglycemic property of the flavonoids isolated from *E. officinalis* was also reported (Anila &

Vijayalakshmi, 2000). *E. littorale* aqueous extract treatment in alloxan-induced diabetic rats showed significant decrease in glycosylated haemoglobin, liver glucose-6-phosphatase activity and significant increase in serum insulin levels of the diabetic rats (Vijayvargia et.al., 2000; Maroo et.al., 2003). It had also been reported to increase insulin sensitivity in streptozotocin-induced NIDDM rats (Murali et.al., 2002). There was also no significant change in the toxic parameters studied (Table I) and hence the extracts were not toxic at the particular dose used.

Moreover the comparison of the different chemical constituents present in *E. littorale* and herbal combination shows an increased presence of all the components as compared to the former (Table II) and thus supporting the increased potency of the combination. The herbal combination has various biologically active components with varied mechanism of action and hence *ex-vivo* experiments with isolated rat pancreatic islets incubated with *E. littorale* and herbal combination for insulin release is an effort in this direction. Both *E. littorale* and herbal combination were incubated with 11.1 mM glucose and insulin release was observed. It was seen that there was a significant increase in insulin release in both treated groups, at 10 and 60 minutes as compared to control group. Islets treated with 20 μg herbal combination showed increased insulin release as compared to other treatment groups (Fig 11). Similarly 4-hydroxy isoleucine isolated from fenugreek seeds had enhanced glucose induced insulin release at 11.1 mM glucose (Sauvaire et al, 1998) and other plants like *Ocimum sanctum* (Nyarko et.al., 2002) and a Siddha drug, Vatharasavangam had shown glucose induced insulin release from isolated rat pancreatic islets (Padmini et.al., 1990). The glucose lowering effect of aqueous extract of *E. littorale* was reported 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).

Thus it can be concluded from the above results that aqueous extract of herbal combination showed good hypoglycemic potential than *E. littorale* alone, without any toxic effect and thus could be a candidate for therapeutic intervention against the many manifestations of diabetes mellitus.

Summary

Herbal combination treatment to alloxan-induced diabetic rats showed an early response in glucose lowering effect from 2nd hr as against a delayed response of *E. littorale* from 4th hr of administration in short term experiments. Moreover the efficacy of the herbal combination was more in decreasing blood glucose and glycosylated haemoglobin levels and increasing serum insulin levels in treated diabetic rats in long term experiments without any significant change in normoglycaemic rats. Thus, demonstrating that the action was glucose dependent.

Serum GPT, ALP and creatinine levels and liver GPT and ALP levels in normoglycaemic and diabetic rats also did not change after extract treatment as compared to their respective controls.

There was an increase in insulin release from isolated rat pancreatic islets in the herbal combination (20 μg) treated group as compared with other groups.

Combining the medicinal plants increased the hypoglycemic potential as compared to *E. littorale* alone and combination therapy must be pursued as a potential candidate against amelioration of diabetes mellitus.

Table I: Effect of *E. littorale* and herbal combination on toxicity parameters

GPT activity (Blood = IU/ml of serum, Liver = IU/g of tissue)

	Untreated	EL treated	ALL treated
Serum			
Normoglycemic	71.90 ± 1.25	74.02 ± 1.93	78.03 ± 5.63
Diabetic	79.95 ± 2.56	77.52 ±5.56	71.24 ± 1.84
Liver			
Normoglycemic	0.99 ±0.05	1.00 ± 0.03	0.86 ± 0.05
Diabetic	1.07 ± 0.04	0.97 ± 0.05	0.95 ± 0.06

ALP activity (Blood = IU/ml of serum, Liver = IU/g of tissue)

	Untreated	EL treated	ALL treated
Serum			
normoglycemic	158.5 ±9.74	136.7 ± 10.66	148.3 ± 5.79
diabetic	204.0 ± 32.91	231.7 ±11.10	190.1 ± 10.71
Liver			
Normoglycemic	0.91 ± 0.03	0.89 ± 0.05	1.00 ± 0.13
diabetic	1.03 ± 0.11	0.92 ± 0.07	0.90 ± 0.06

Serum creatinine (mg/dl)

	Untreated	EL treated	ALL treated
normoglycemic	1.02 ± 0.07	1.00 ± 0.05	0.93 ± 0.07
diabetic	1.07 ± 0.09	0.91 ± 0.09	1.07 ± 0.10

Values are expressed as mean ± SE (n = 5 – 6)

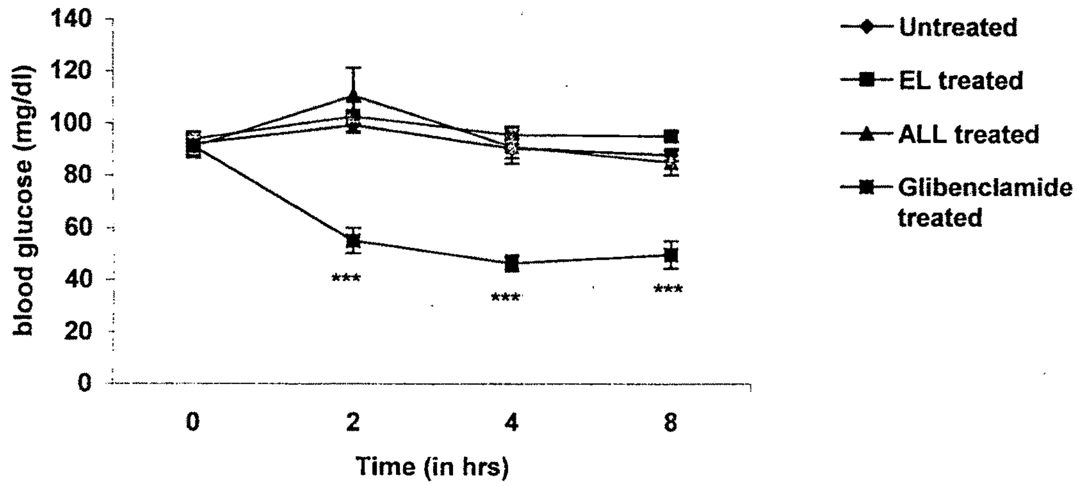
Table II: Qualitative analysis of the aqueous extract of *Enicostemma littorale* Blume and Herbal combination ***(C. longa, E. officinalis, T. foenum-graecum, E. littorale)***

Class of compounds	*Enicostemma littorale* Blume aqueous extract	Herbal combination aqueous extract
Phenolic	++	+++
Tannins	+++	+++
Anthroquinone	++	+++
Flavonoids	++	+++
Glycosides	+++	+++
Saponins	-	++

++ - present in low to moderate levels

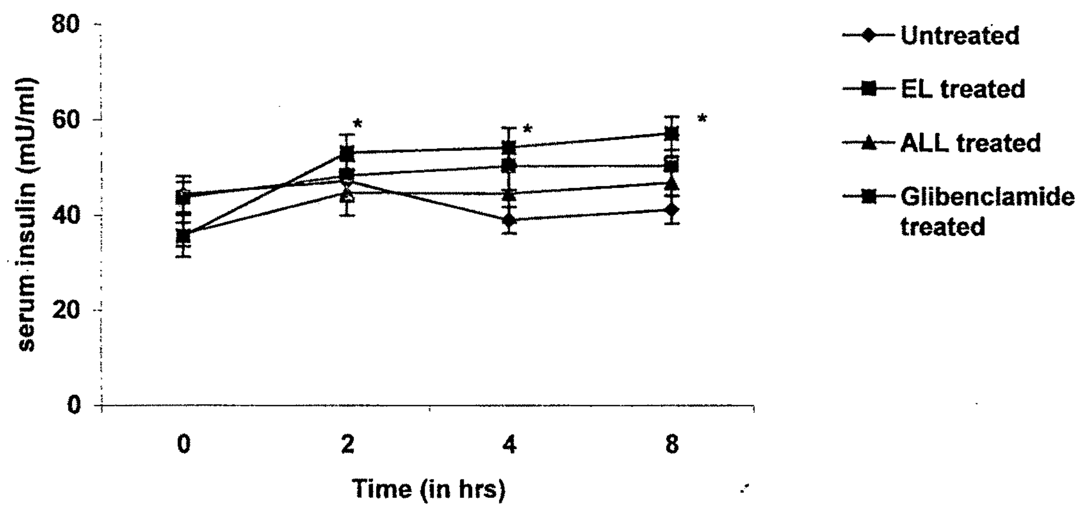
+++ - present in high levels

Fig 1: Effect of *E. littorale* (EL) aqueous extract and herbal combination (ALL) on blood glucose levels in normoglycemic rats



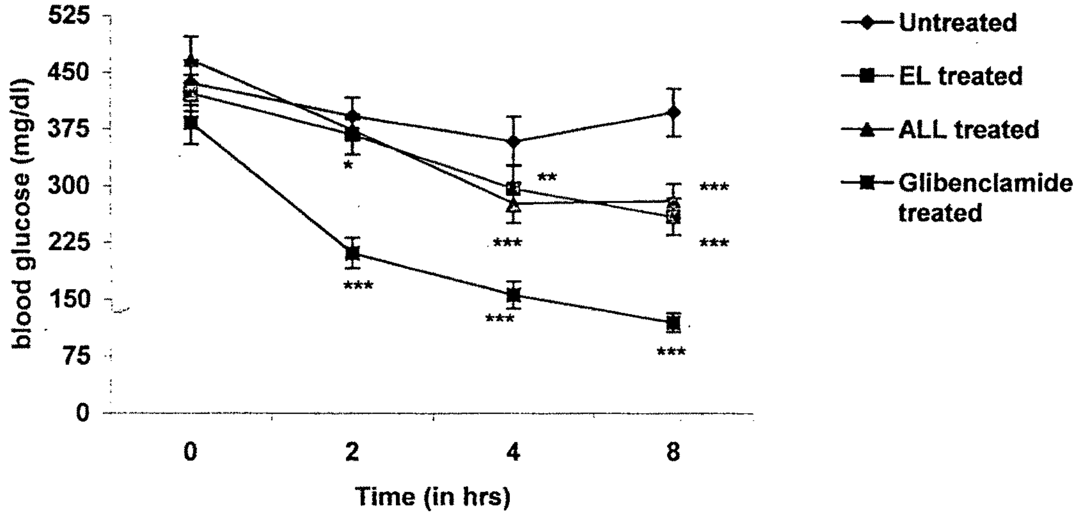
*** P< 0.001 as compared to 0 hr values

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



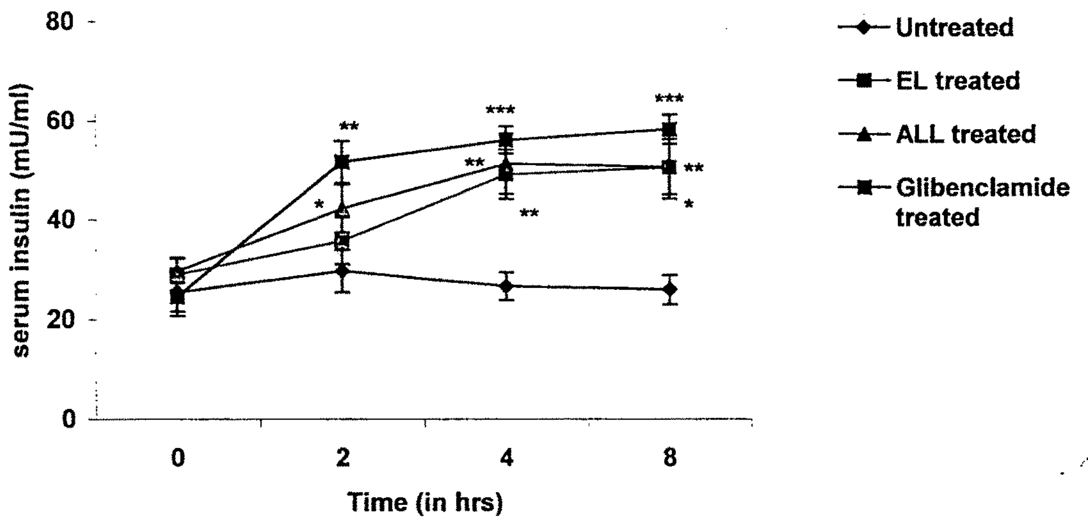
* P< 0.05 as compared to 0 hr value

Fig 3: Effect of *E. littorale* (EL) aqueous extract and herbal combination (ALL) on blood glucose levels in alloxan-induced diabetic rats



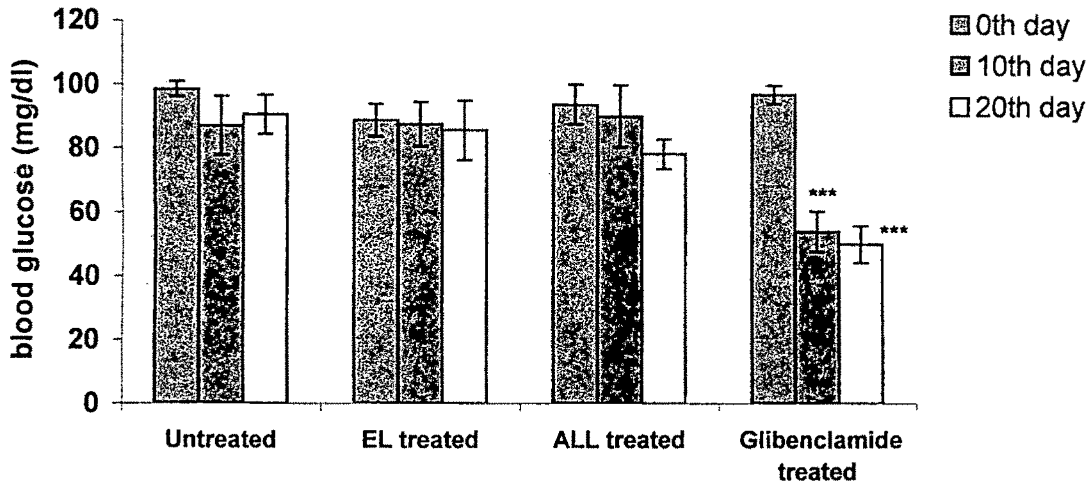
* P< 0.05, ** P< 0.01, *** P< 0.001 as compared to 0 hr value

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



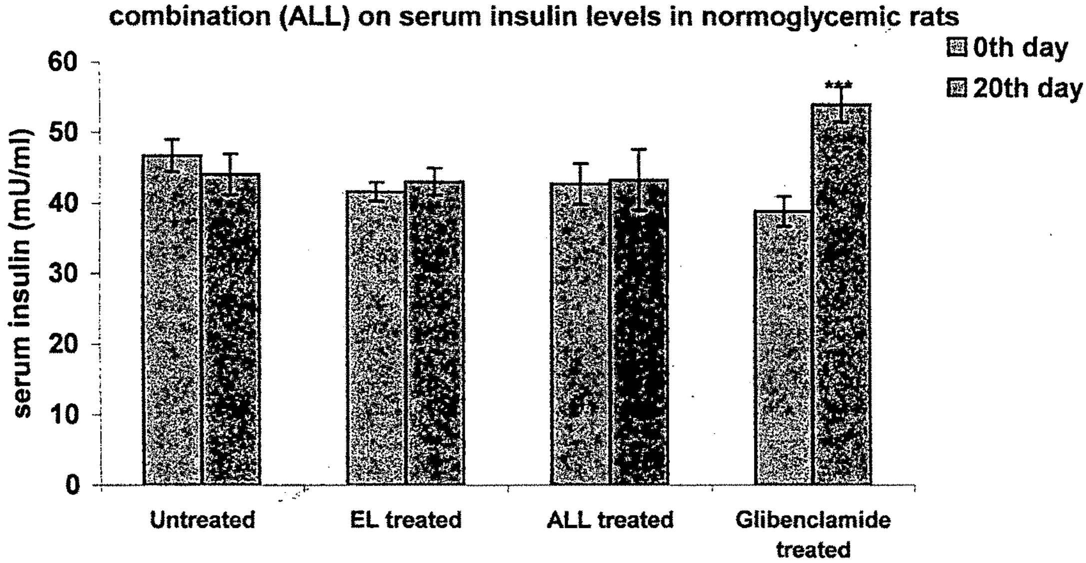
* P< 0.05, ** P< 0.01, *** P< 0.001 as compared to 0 hr value

Fig 5: Effect of aqueous extract of *E. littorale* (EL) and herbal combination (ALL) on blood glucose levels in normoglycemic rats



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

Fig 6: Effect of aqueous extract of *E. littorale* (EL) and herbal combination (ALL) on serum insulin levels in normoglycemic rats



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

Fig 7: Effect of aqueous extract of *E. littorale* (EL) and herbal combination (ALL) on glycosylated haemoglobin levels in normoglycemic rats

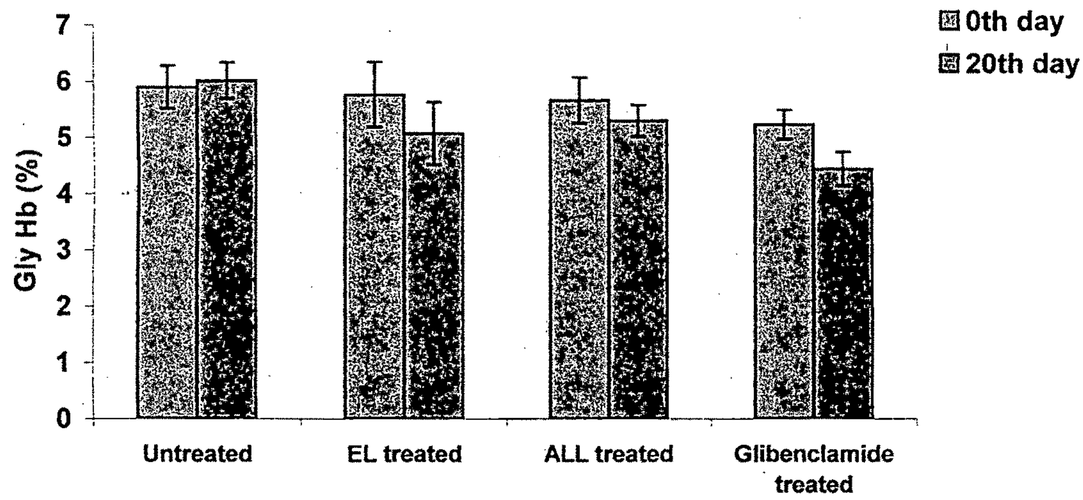
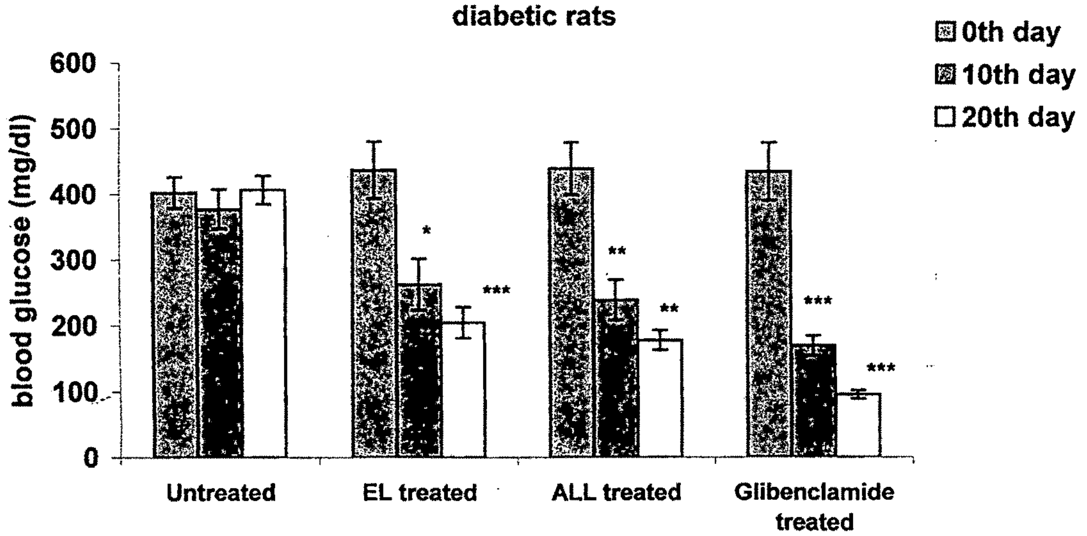
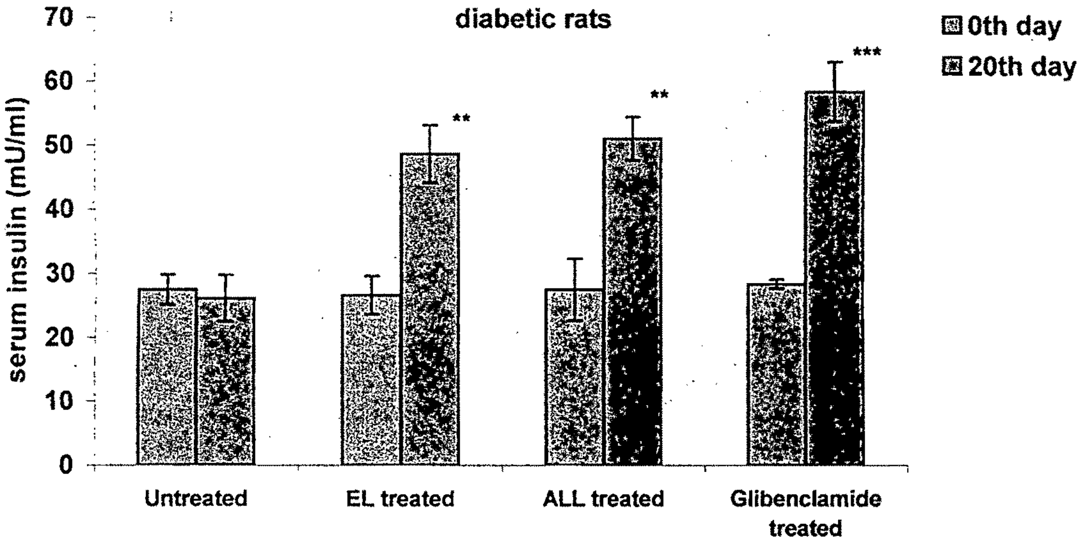


Fig 8: Effect of aqueous extract of *E. littorale (EL)* and herbal combination (ALL) on blood glucose levels in alloxan induced diabetic rats



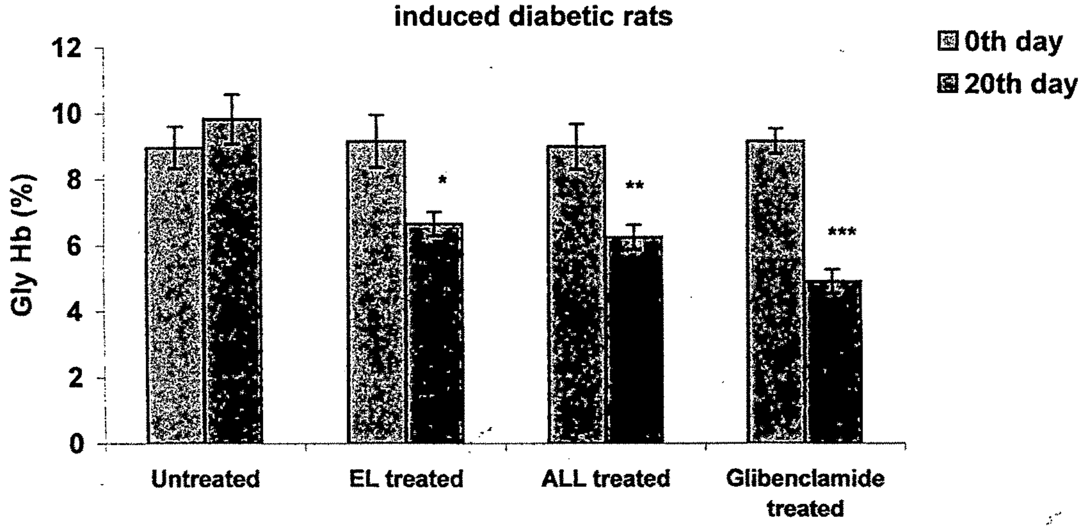
* P< 0.05, ** P< 0.01, *** P< 0.001 as compared to 0th day value

Fig 9: Effect of aqueous extract of *E. littorale* (EL) and herbal combination (ALL) on serum insulin levels in alloxan-induced diabetic rats



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

Fig 10: Effect of aqueous extract of *E. littorale* (EL) and herbal combination (ALL) on glycosylated haemoglobin levels in alloxan-induced diabetic rats



* P< 0.05, ** P< 0.01, *** P< 0.001 as compared to 0th day value

Fig 11: Effect of aqueous extract of *E. littorale* (EL) and herbal combination (ALL) on glucose-induced insulin release from isolated rat pancreatic islets