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Hypoglycemic effect of a polyherbal aqueous extract in experimentally induced diabetic rats

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SUMMARY

The present study was carried out to investigate the hypoglycemic effect of a polyherbal aqueous extract (*Curcuma longa Linn., Emblica officinalis Gaertn., Trigonella foenum-graecum Linn., Enicostemma littorale* Blume) in alloxan-induced diabetic rats. Short term experiments showed a decrease in blood glucose levels at 2nd hr of administration of the aqueous extract in alloxan-induced diabetic rats with increase in serum insulin levels. The extract did not show any effect on blood glucose or serum insulin levels in normoglycaemic rats. Treatment with the extract (1.5 g dry plant equivalent extarct/100 g body weight/day) for 20 days in diabetic rats showed a significant decrease in blood glucose and glycosylated haemoglobin levels and an increase in serum insulin levels. The aqueous extract also showed an enhanced glucose-induced insulin release at 11.1 mM glucose from isolated rat pancreatic islets. The extract did not show any toxicity at the particular dose used.

Key words: *Curcuma longa Linn.; Emblica officinalis Gaertn.; Trigonella foenum-graecum Linn.; Enicostemma littorale* Blume; Hypoglycaemic; Rat pancreatic islets; Insulin secretion

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). The unidirectional therapeutic approach in the management of diabetes does not appear to be the way to address this problem. Most of the antidiabetic herbal preparations or "Ayurvedic medicines" available in the market are combinations of various medicinal plants. 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.

A polyherbal combination of *Curcuma longa* Linn. dry rhizome, *Emblica officinalis* Gaertn. dry fruit, *Trigonella foenum-graecum* Linn. dry seeds and whole dry plant of *Enicostemma littorale* Blume, are widely being used as an antidiabetic agent by quacks in Gujarat, India. The effect of *C. longa* or turmeric and its active principle curcumin was found to significantly decrease blood sugar, and glycosylated hemoglobin levels (Arun and Nalini, 2002). The hypoglycemic and hypolipidaemic properties of the flavonoids of *Emblica officinalis* or amala were reported by Anila and Vijayalakshmi. Blood glucose was reduced by an aqueous extract of Trigonella leaf in rats whereas ethanolic extract was only active by ip administration (Abdel-Barry

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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, b) in alloxan-induced diabetic rats and diabetic patients (Vasu *et al.*, 2003) 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 (Murali *et al.*, 2002) and preventing the progression of complications in Type 2 diabetic patients were also reported by other workers (Upadhyay and Goyal, 2004).

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

MATERIALS AND METHODS

Extract preparation

Whole dried plant of *Enicostemma littorale* Blume, dried rhizome of *Curcuma longa* Linn., dried fruit of *Emblica officinalis* Gaertn. and dried seeds of *Trigonella foenum-graecum* Linn. were procured from the local market and identified and authenticated at the Botany department, M.S. University of Baroda, Gujarat, India. They were taken in equal proportions, crushed, soaked and boiled twice for 30 minutes. The filtrates were combined and evaporated to obtain the desired concentration (1 g dry plant equivalent extract/ml). The yield of the extract was found to be 24%. This was used as aqueous extract in the study.

Experimental design

Experiments were carried out both *in-vivo* and ex-vivo. *In-vivo* experiments were further divided into – short-term and long-term experiments. 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/100 g body weight) of herbal combination (ALL), and the untreated control rats received equal amount of tap water via gastric intubation. Glibenclamide (0.25 mg/100 g 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 (1.5 g dry plant equivalent extarct/100 g body weight/day) of herbal combination (ALL) 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 haemoglobin (Gly Hb) (Parker *et al.*, 1981) and serum insulin levels on 0^{th} and 20^{th} day. Serum glutamyl pyruvate transaminase (SGPT) (Reitman and Frankel, 1957), serum alkaline phosphatase (ALP) (Bowers and McComb, 1975), serum creatinine levels (Henry *et al.*, 1974) 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 (ALL) by incubating the extract with isolated rat pancreatic islets. Rat pancreatic islets were isolated from the pancreas by collagenase digestion (Xia and Laychok, 1993). These islets were picked up manually in a batch of 10 islets under stereomicroscope in each tube for all the experiments. Further, in all the *in vitro* experiments islets were preincubated with 0.5 ml incubation buffer containing 11.1 mM glucose alone or in combination with concentrations of the polyherbal aqueous extract (10 and 20 µg dry plant equivalents) at 37°C for 30 min in a shaking waterbath. After the preincubation was over, buffer was removed and all the additions were done in similar fashion and incubated under similar conditions. Aliquots of 50 µl were removed from static incubation mixture at 10 and 60 min, and were frozen immediately until insulin assay was performed using RIA.

Statistical analysis

Statistical evaluation of analytical data was done by Student's *t* test using the statistical software-GraphPad Prism 3.0. In all comparisons two-tailed $P \leq 0.05$ was considered significant. Results are expressed as mean ± SE.

RESULTS

Short-term experiment

In the short term experiments, administration of aqueous extract of the herbal combination 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 an 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 and 2). In alloxan-induced diabetic rats, herbal combination showed blood glucose lowering effect from 2nd hr of administration (Fig. 3). The results were similar with regard to serum insulin levels (Fig. 4).

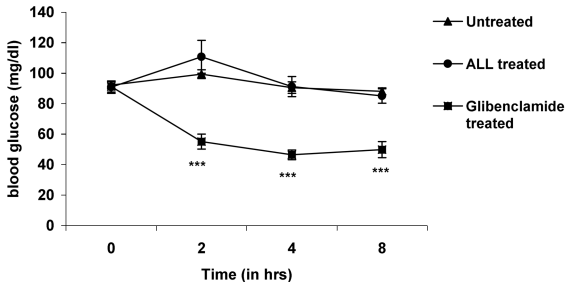

Fig. 1. Time dependent effect of herbal combination aqueous extract on blood glucose levels in normoglycemic rats. ***$P < 0.001$ as compared to 0th hr. Values presented as mean ± SE (n = 6).

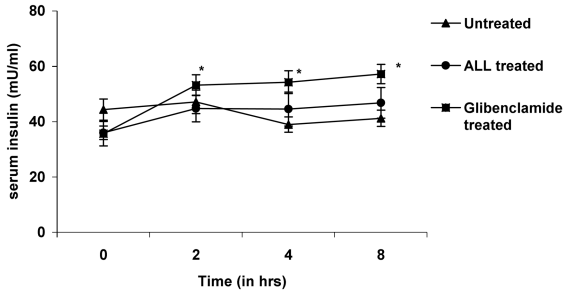

Fig. 2. Time dependent effect of herbal combination aqueous extract on serum insulin levels in normoglycemic rats. *$P < 0.05$ as compared to 0th hr. Values presented as mean ± SE (n = 6).

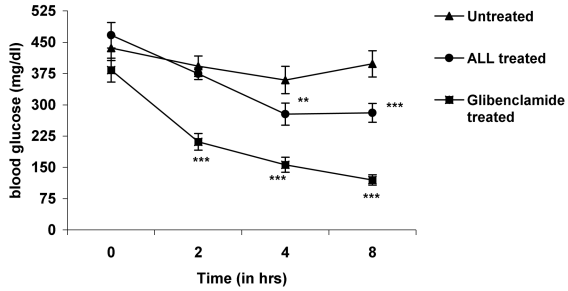

Fig. 3. Time dependent effect of herbal combination aqueous extract on blood glucose levels in alloxan-induced diabetic rats. **$P < 0.01$, ***$P < 0.001$ as compared to 0th hr. Values presented as mean ± SE (n = 6).

Long-term experiment

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 (Data

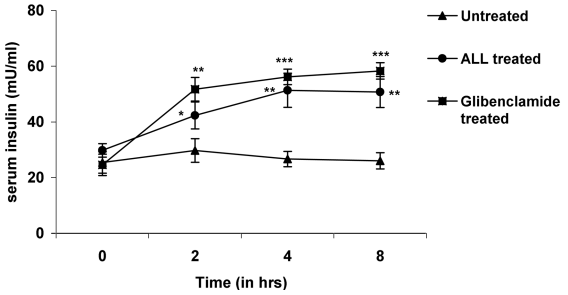

Fig. 4. Time dependent effect of herbal combination aqueous extract on serum insulin levels in alloxan-induced diabetic rats. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ as compared to 0^{th} hr. Values presented as mean ± SE (n = 6).

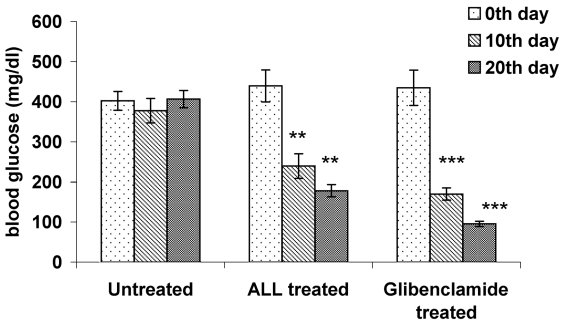

Fig. 5. Effect of herbal combination aqueous extract on blood glucose levels in alloxan-induced diabetic rats. $^{**}P < 0.01$, $^{***}P < 0.001$ as compared to 0^{th} day value. Values presented as mean ± SE (n = 6).

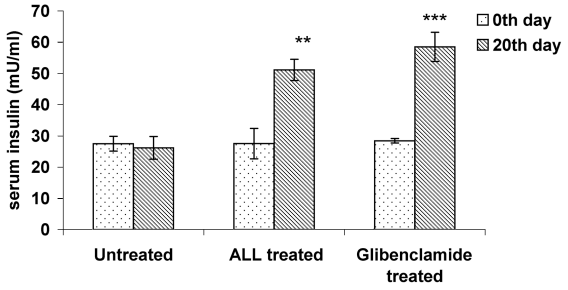

Fig. 6. Effect of herbal combination aqueous extract on serum insulin levels in alloxan-induced diabetic rats. $^{**}P < 0.01$, $^{***}P < 0.001$ as compared to 0^{th} day value. Values presented as mean ± SE (n = 6).

not shown). But there was a significant decrease in blood glucose, glycosylated haemoglobin levels and an increase in serum insulin levels in herbal combination aqueous extract treated diabetic rats as compared to 0^{th} day values (Fig. 5 - 7). The

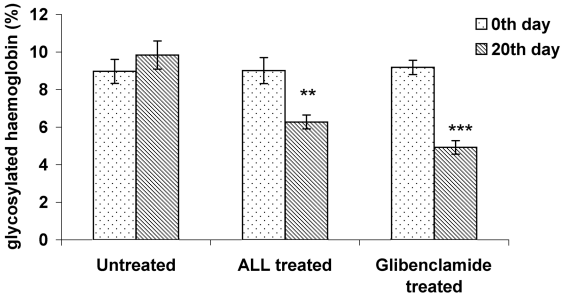

Fig. 7. Effect of herbal combination aqueous extract on glycosylated haemoglobin levels in alloxan-induced diabetic rats. $^{**}P < 0.01$, $^{***}P < 0.001$ as compared to 0^{th} day value. Values presented as mean ± SE (n = 6).

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 alloxan-induced diabetic rats (Table 1).

Ex-vivo experiment

Ex-vivo experiments with aqueous extract of 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 mg concentrations as compared to untreated control (Fig. 8).

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/ 100 g body weight was used for the study, as this was found to be the effective dose with regard to preliminary experiments conducted in the lab (Data not shown). Aqueous extract of herbal combination when administered to normoglycaemic rats for 8 hrs did not show any hypoglycemic

Table 1. Effect of aqueous extract of the herbal combination (ALL) on serum and liver GPT, ALP and serum creatinine levels in alloxan-induced diabetic rats at 20th day.

	Untreated	Treated
Serum GPT (IU/L serum)		
Normoglycaemic	71.90 ± 1.25	78.03 ± 5.63^{NS}
Diabetic	79.95 ± 2.56	71.24 ± 1.84^{NS}
Liver GPT (IU/ g tissue)		
Normoglycaemic	0.99 ± 0.05	0.86 ± 0.05^{NS}
Diabetic	1.07 ± 0.04	0.95 ± 0.06^{NS}
Serum ALP (IU/L serum)		
Normoglycemic	158.5 ± 9.74	148.3 ± 5.79^{NS}
Diabetic	204.0 ± 32.91	190.1 ± 10.71^{NS}
Liver ALP (IU/g tissue)		
Normoglycemic	0.91 ± 0.03	1.00 ± 0.13^{NS}
diabetic	1.03 ± 0.11	0.90 ± 0.06^{NS}
Serum Creatinine (mg/dl)		
Normoglycemic	1.02 ± 0.07	0.93 ± 0.07^{NS}
Diabetic	1.07 ± 0.09	1.07 ± 0.10^{NS}

(IU = μM product formed/min)
P = NS (non-significant) as compared to untreated groups
Values are expressed as mean ± SE (n = 6)

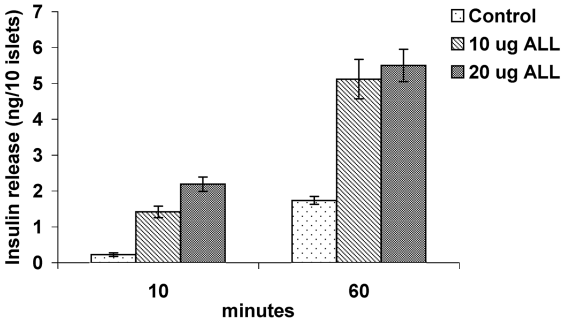

Fig. 8. Effect of herbal combination aqueous extract on glucose induced insulin release from isolated rat pancreatic islets. Values presented as mean ± SE (n = 3).

effect. But there was a significant decrease in blood glucose levels in glibenclamide treated normoglycaemic rats. When the extract was administered to alloxan-induced diabetic rats, herbal combination showed a significant decrease in blood glucose levels from the 2nd hr of administration. The above results showed that 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 herbal combination treated normoglycaemic rats, which were supportive of the short term experiments. In alloxan-induced diabetic rats, 20 days treatment 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 0th day. 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, 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 and Vijayalakshmi, 2000). *E. littorale* aqueous extract treatment in alloxan-induced diabetic rats showed significant decrease in glycosylated haemoglobin, liver glucose-6-phosphatase activity, fructose 1,6–bisphosphatase and significant increase in serum insulin levels and liver hexokinase activity of the diabetic rats (Vijayvargia *et al.*, 2000; Maroo *et al.*, 2003b; Srinivasan *et al.*, 2005). 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 1) and hence the extract showed no toxicity at the particular dose used.

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 herbal combination is an effort in this direction. Herbal combination was 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 (Fig. 8). 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 canum* (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 potent hypoglycemic potential by way of inducing insulin release, and thus could be a potential candidate for therapeutic intervention against the many manifestations of diabetes mellitus.

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